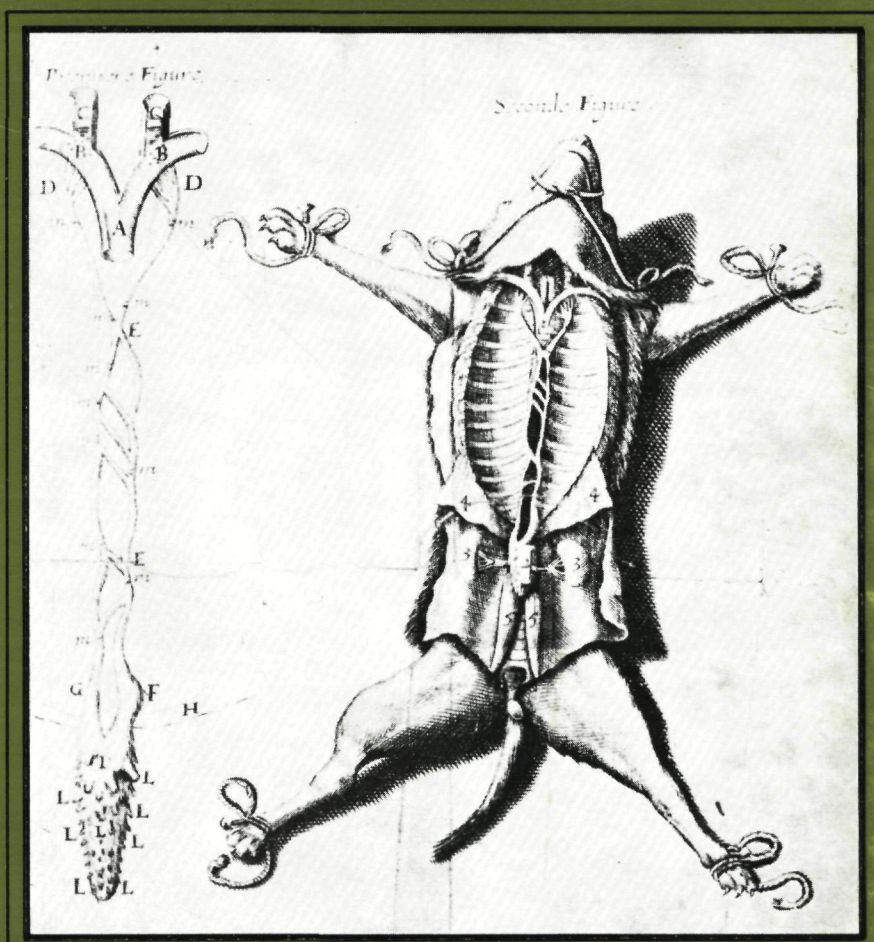
participation of the lymphatic system in the absorption of pharmaca from the gut



h.kilian

Cover thoracic duct and receptaculum chyli, by Jean Pecquet (1651) Experimenta Nova Anatomica

PARTICIPATION OF THE LYMPHATIC SYSTEM IN THE ABSORPTION OF PHARMACA FROM THE GUT

PROMOTORES

PROF DR E J ARIËNS

EN

PROF DR J M DENUCÉ

PARTICIPATION OF THE LYMPHATIC SYSTEM IN THE ABSORPTION OF PHARMACA FROM THE GUT

PROEFSCHRIFT
TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE
WISKUNDE EN NATUURWETENSCHAPPEN
AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. MR. F. J. F. M. DUYNSTEE,
VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN
IN HET OPENBAAR TE VERDEDIGEN OP
DONDERDAG 15 MAART 1973, DES NAMIDDAGS TE 16.00 UUR

DOOR

HENDRIK KILIAN

GEBOREN TE UTRECHT

DRUKKERIJ BRAKKENSTEIN TE NIJMEGEN

These investigations were carried out in the Institute of Pharmacology, University of Nijmegen, the Netherlands, and have been supported by grants from the Dr. Saal van Zwanenberg Foundation and the Dutch Organization for Purely Scientific Research (FUNGO - ZWO).

aan Dorine

die zoveel moest doen

voor Kitty, Francis,
Dorientje en Lizet

die zoveel moesten laten

ACKNOWLEDGEMENT

The author is greatly indebted to all persons who contributed to the realization of this study. He wishes to thank the whole staff of the Institute of Pharmacology of the University of Nijmegen for support and assistance, and for many valuable suggestions and criticism.
The technical assistance of Mr. A. C. Wouterse, Miss N. J. W. M. Hoedemaeckers, Mr. F. W. Barten and Miss J. H. J. van Breda is gratefully acknowledged.
Thanks are also due to Mr. P. B. Spaan (Central Animal Laboratory, head: Dr. M. J. Dobbelaar) for his skilful assistance in the performance of the animal experiments. Many thanks are due to Mr. C. P. Nicolasen for drawing the figures and designing the cover.
The author also wishes to thank: Dr. Th. J. Benraad, Drs. T. J. Exler (L.N.A., The Hague), Dr. H. J. Hoenders, Dr. A. P. Jansen, Drs. J. F. Rodriguez de Miranda, Dr. J. H. Veerkamp, Dr. J. M. C. Wessels and Drs. C. C. B. Wijffels for assisting and/or supplying materials; Dr. P. Th. Henderson and Dr. E. M. Aarts for their constructive remarks in the preparation of the manuscript; Dr. R. de Graaf for his assistance in the statistical evaluation of the results; Mr. E. de Graaff and co-operators and Miss D. J. A. Thuis for bibliographic aid; Drs. W. W. K. Cotton for polishing the English text, and many others for their more incidental assistance and remarks. Prof. Dr. C. A. Salemink (Org. chem. lab. University of Utrecht, the Netherlands) is thanked for the gift of Δ 1-tetrahydrocannabinol; Ir. R. H. de Vos (Central Institute for Nutrition and Food-research, TNO, Zeist, the Netherlands) for gifts of hexachlorbiphenyl and aroclor and for his assistance in analyzing these compounds in lymph samples. Gist-Brocades N.V. (Delft, the Netherlands) is thanked for gifts of ^{14}C labelled and unlabelled cyclandelate; ICI Holland N.V. (Rotterdam, the Netherlands) for the gift of butylated hydroxytoluene; Merrell Division (London, England) for the gift of chlorotrianisene; N.V. Organon (Oss, the Netherlands) is thanked for gifts of ^{14}C labelled und unlabelled lynestrenol, mestranol, nandrolone and progesterone and for unlabelled α-tocopherol alcohol, vitamin K_1 and β-sitosterol; N.V. Philips-Duphar (Weesp, the Netherlands) for gifts of ^{14}C labelled vitamin D_3 and for unlabelled 25-HCC; Unilever-

Emery N.V. (Gouda, the Netherlands) for the gift of triolein; Instituto Warner-Vister (Casatenovo, Italy) for gifts of ethinylestradiol 3-cyclopentyl ether, progesterone 3-enol cyclopentyl ether and 17 α-methyltestosterone 3-enol cyclopentyl ether. Both quinestrol and quinestrol-6.7-^{3}H were provided by the Warner-Lambert Research Institute, Morris Plains, N.J., research affiliate of Warner-Chilcott Laboratories.

CONTENTS

CHAPTER 1

1.1. INTRODUCTION

Many drugs absorbed from the gut are taken up in the blood of the portal system and transported to the liver. This is the usual idea about the absorption process of drugs taken orally. However the body has not only a vascular system but also a lymphatic system that may function in the absorption process. The question arises whether the latter system plays an important role in the absorption of drugs or not. After a meal, fat components are transported in the chyle, whereby this becomes white. The lymph vessels can be traced, by this white colour, from the gut, via the cisterna chyli and the thoracic duct, to its ending in the neck where it arches laterally to open near, or at, the angle of union of the internal jugular and subclavian veins (Yoffey and Courtice, 1956; Miotti, 1965; Boileau Grant, 1962) (see fig. 1). In fact absorbed drugs could also follow this route.

Up to now only a few drugs and nutrients are known to be absorbed to a large extent via the lymphvessels. For instance, a considerable absorption via the lymphvessels is reported for the cyclopentylether of ethinylestradiol = quinestrol (Giannina et al, 1967) and DDT (Rothe et al, 1957). Fat soluble vitamins are generally absorbed to a large extent via this route, e.g. vitamin A (Huang and Goodman, 1965), vitamin D_3 (Schachter et al, 1964), vitamin E (Blomstrand and Forsgren, 1968a), vitamin K_1 (Blomstrand and Forsgren, 1968b). Also the long chain fatty acids either in the free form or as triglycerides (Hyun et al, 1967) and cholesterol (Chaikoff et al, 1952; Schachter et al, 1964) are absorbed via the lymphvessels.

Our investigations were started to answer the following questions:

- Which drugs are absorbed to a great extent via the lymphatics?
- What are the properties that lead to such an absorption?
- Is it possible to directly influence the absorption in the direction of the portal system or the lymphatics by means of the vehicle?

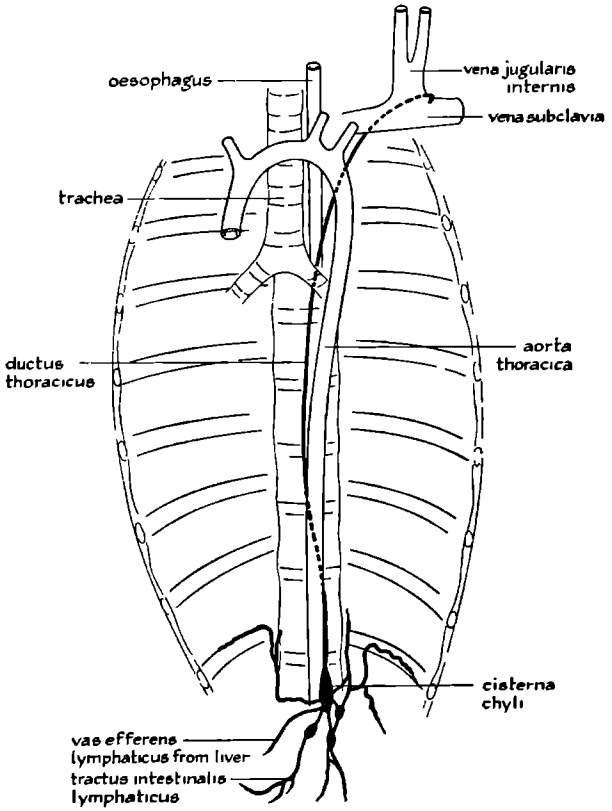


Fig. 1
Route of lymph tract from intestine to the angle of union of the internal jugular and subclavian vein (after Miotti, 1965).

- Are there pharmacologic consequences of influencing the route of absorption?

Concerning the last question the following facts may be relevant. If a drug is absorbed exclusively via the portal system then it goes directly to the liver. The liver has a great capacity for metabolizing, and eventually for inactivating, a drug. If, on the other hand, a drug is absorbed exclusively via the lymphatic tract of the gut, then this drug will be transported into the blood of the vena subclavia. In this case the drug may reach its site of action or may be trapped in the fat depots whithout passing through the liver. So, a difference in intensity

and duration of action of a drug may occur if it is absorbed via one route rather than another.

In this respect it is clear that the following aspects are of importance:
A. What properties must a drug have and/or which factors are necessary for it to be absorbed in a much greater concentration via the lymphatics than via the portal system? According to Landis (1962) both the blood capillaries and lymphatic vessels are freely permeable to all small molecular weight compounds and to all lipid-soluble compounds of moderate size. For such compounds the blood capillaries constitute the major route of absorption as a result of rapid blood flow. This idea was clearly stated by Hendrix and Sweet (1917): "it is suggested that the practically complete absorption of protein and carbohydrate by the blood is not due to a selective absorption, but to the almost infinitely large volume of blood, as compared to the volume of lymph, which flows through the walls of the intestine."
Bollman et al (1948a) found that about 80 per cent of the thoracic duct lymph of the rat was derived from the intestinal tract and about 20 per cent from the liver. The flow rates for the thoracic duct lymph, therefore, give a reasonable estimate of intestinal lymph flow under most conditions. Portal blood flow in each case is approximately 500 to 1000 times that of lymph. The flow rates on the basis of body weight are remarkably similar in the rat, dog and human (Landis, 1962).
If such compounds as glucose and amino acids entered both types of vessels with equal ease, over 99 per cent would be absorbed through the portal vein. It implies that only if the lymph absorbs a much greater amount of the compound than the portal system, will the transport in lymph be of quantitative importance.

B. Which are the properties of drugs and what factors are necessary so that the molecules. which are absorbed from the gut in the lymphatics, will stay there until they pass into the vena subclavia?
Levine and coworkers (1969) investigated a number of drugs in respect of their absorption via blood and lymph. Soon after administration all the different kinds of compounds studied were found to be present in blood and lymph in nearly the same concentrations. These results suggest that molecules will interchange between the blood and the interstitial tissue and lymph. If molecules demonstrate an exchange balance between blood and lymph, then, because of the slow flow of the lymph, the molecules which are transported in lymph tracts to the

vena subclavia, will have previously passed through the liver. So the idea that molecules escape metabolism by the liver may only hold true if there is no exchange balance.

In relation to the points in sections A. and B. the following facts are of importance. The lymph tract wall has the astonishing property of letting particles with molecular weights greater than anywhere from 2300 to 6000 in with great ease, but not letting them out (Mayerson, 1968). Compounds that are transported in considerable amounts by the lymph, like the fat soluble vitamins and DDT, have low molecular weights, but are transported in lymph together with fat. This fat is transported mainly in the form of chylomicrons. These are relatively large colloidal fat globules, of 200 to 300 nm diameter, which are produced in the intestinal mucosa. It is known that for 80 per cent or more the fat soluble vitamins and DDT are present in chylomicrons. So if small molecules with a molecular weight greater than 6000 bind tot chylomicrons the resulting complexes are transported in the lymphatics and thus the small molecules stay there. These facts may answer the questions posed. If one wants to detect whether drugs are transported via the lymphatics in considerable amount, the lipophilic character of such drugs could be of importance. Moreover the administration in fatty preparations may be decisive. Significant absorption via lymph tracts of compounds that bind strongly to protein molecules has not, up to now, been reported.

C. Could the pharmacologic consequences of influencing the route of absorption be connected with fat kinetics? DDT and the fat soluble vitamins arrive in the blood of the vena subclavia bound to chylomicrons. Chylomicrons display a characteristic behaviour. They disappear very fast from the blood with an apparent half-life of only a few minutes. It is an interesting question to what extent compounds bound to chylomicrons follow these events.

Other aspects may be considered.
Some natural steroids like testosterone and nandrolone are decomposed by the liver when given orally (Biskind and Mark, 1939; Biskind, 1940; Kupperman, 1965). For that reason no pharmacologic effect is achieved. Alibrandi et al (1960) investigated some steroid compounds and concluded that all compounds tested exhibited an increase in specific activity when administered orally in oily solution instead of

aqueous suspension. The question now arises whether the increase in specific activity is the result of an enhanced lymphatic transport due to the use of oil as vehicle.
Rothe et al (1957) looked for lymphatic absorption of DDT and its metabolites in the rat. About 60 per cent of the dose of DDT, which was not lost in the intestinal contents and faeces, was found in the chyle. They could not account for the remaining 40 per cent but they state: "Because of the diffuse, poorly defined nature of the lymphatic system, collaterals which bypassed the cannulated duct are a real possibility. It seems likely therefore that most, if not all, of the DDT derived materials are absorbed by way of the lymphatic system, but the possibility of some absorption by way of the hepatic portal system can not be excluded by these experiments."
So these investigators believe in nearly a hundred per cent absorption of DDT via the lymphatic system. Also the transport of cholesterol has always been regarded as occurring exclusively via the lymphatic pathway (Treadwell and Vahouny, 1968). However, recent results of MacMahon et al (1971) indicate that under certain circumstances, a significant proportion of cholesterol and also vitamin E may be absorbed via the portal vein.
To what extent lymph is transported by collaterals from the lymphatic system to the vascular system has been studied in rats by Threefoot et al (1963). They visualized the lymphatic vessels by the injection of coloured plastic. In those animals in which the cisterna chyli was not ligated or was ligated immediately before the injection of the plastic, no additional lymphatico-venous communications were observed. Only when the cisterna chyli was ligated 21-32 days beforehand, lymphatico-venous communications were found. So it is likely, that there is an enlargement of pre-existing subsidiary channels which under normal circumstances carry little or no lymph. In the present investigations no further attention was paid to the role of collaterals in the lymphatic transport.

1.2. THE STRUCTURE OF THIS THESIS

The aim of this investigation is to gather more information about the questions posed. For that reason the following study was conducted.

In chapter 2 a description of the materials, animal experiments, and analytical procedures used in this study is given.

Chapter 3 describes the influence of dosage forms (i.e. triolein, micellar solution and propylene glycol), partly on rats with bile cannulation, and on lymphatic or portal absorption of DDT. The degree to which fat absorption is important for the transport of DDT via the lymph duct was investigated; a form of administration was sought which would be optimal in this respect; also the influence of bile on this process was examined.

Chapter 4 gives 1. The amount of various compounds (radioactively labeled) collected in the thoracic duct lymph at different intervals up to 24 hr after oral administration. The amount in a number of excretion and organ samples during and after 24 hr was determined. In a number of cases the effects of both fat and propylene glycol administration were studied. The extent of lymphatic transport dependent on the site of administration was also studied.
2. Ratios of concentrations of various drugs in mesenteric lymph to portal blood during absorption. In the case of a number of drugs the total amount of radioactivity was studied as was the absorption of the pure drug.
3. Determination of lipophilicity as ΔR_m values against butter yellow. With the aid of reversed phase chromatography these values were determined.
4. The extent to which the lipophilicity of the drugs determined the transport in the lymph was examined. 24 hr absorptions were correlated with ΔR_m values.

In chapter 5 the results are discussed of studies concerning whether and to what extent binding of drugs to chylomicrons could play a role; to what degree binding to other lipoproteins and to other proteins occurs; the extent to which the drugs are bound to nucleus or skin of the chylomicrons under various conditions. Therefore binding studies were carried out with vitamin D_3, DDT, dieldrin, quinestrol and mestranol to lymph components under various conditions.

Chapter 6: By measuring the amount and degree of conversion of DDT and aldrin in fat and liver samples at various times, we attempt to get an impression of the differences in distribution and metabolism in the body as a result of altered transport via portal vein or lymph tract. Therefore the influence of fatty or non-fatty dosage forms on fat/liver concentration quotient and degree of metabolism in fat of DDT and aldrin was investigated.

Chapter 7: Conclusions and summary.

CHAPTER 2

EXPERIMENTAL PROCEDURES

2.1. ANIMALS

The animals used were rats. It is known that rats have great resistance to infections and post-operative shock (Lambert et al, 1965). This is, for many investigators including ourselves, the main reason for using these animals for lymph cannulations. The animals used were male Wistar rats, weighing 210-230 g.

2.2. OPERATION TECHNIQUE

2.2.1. Narcosis

Operation was done under halothane-nitrous oxide narcosis. This was carried out with the "Loosco T.I.L.C. Fluothane vaporiser". This narcosis is easy to handle for long operations by keeping the N_2O and O_2 flow minimal, e.g. 1 l O_2, 0.5 l N_2O. The mixture contains approx. 0.8 per cent fluothane.

2.2.2. Cannulation

Polythene cannulae were used for all cannulations except in the case of bladder cannulations where polyvinyl chloride was used. The cannulae were supplied by Talas Company, The Netherlands.

2.2.2.1. Lymph cannulations

References to lymph cannulation are given by the following authors: Bollman et al (1948a); Gallo-Torres and Neal Miller (1969); Yoffey and Courtice (1970); Lambert et al (1965). In the cannulation of the thoracic duct and the mesenteric duct we followed the methods of Bollman et al (1948a).

2.2.2.2. Stomach cannulation

A cannula was inserted in the upper part of the stomach with a ligature

according to Lambert et al (1965). This stomach cannula was used for an infusion that contained 0.9 per cent NaCl and 0.04 per cent KCl (Sylvèn and Borgström, 1968).

2.2.2.3. Cannulation of the urinary bladder.
A cannula was inserted in the urinary bladder in order to avoid contamination of urine with faeces.

2.2.2.4. Bile fistula
Under certain circumstances, as indicated elsewhere, bile fistula was employed according to the method of Lambert et al (1965). The cannula is inserted in the upper part of the bile duct, so that the pancreatic juice that leaves the bile duct via the lower part is not obstructed. With this method a bile delivery of 48 hr could be collected (Gallo-Torres et al, 1969). The bile cannula was conducted to the outside via the incision in the abdomen. All other cannulae were conducted via the back under the skin and left the body near the tail.

2.2.2.5. Cannulation of portal vessels.
A cannula was inserted in one of the branches of the portal vein. It is obviously advantageous to cannulate one branch of the portal tract, since only a part of the portal vein is obstructed and clotting occurs only in this cannulated branch. Moreover the liver lobe is also supplied with blood via the arteria hepatica. Before insertion the cannula was filled with saline containing heparin 1 : 25. The portal cannula was connected with an infusion of saline containing heparin 1 : 100. The infusion had a flow of 0.16 ml/hr. So very dilute heparin was delivered at the site of insertion in order to prevent local clotting. The rats survived this portal branch cannulation for up to one day.

2.3. EXPERIMENTAL DESIGN

2.3.1. Restraining cage
After the cannulation procedure the rats were placed in a restraining cage. A modified model of the cage of Bollman (1948b) was used (see fig. 2). Front and back are made of perspex, between these sides are copper bars. The four upper bars can be removed. In this manner a rat can be held in the cage. The inside sizes of the cage are l. 16.0 cm, br. 4.5 cm, h. 4.5 cm. Cages of these sizes are suitable for a rat of about 220 g. A plastic funnel could be inserted through

the back of the cage and attached to a plastic container. The container stood in ice water. The vessels carrying the other samples were also cooled in this way. Via a slit in the front of the cage the rat had free access to water. The surrounding air temperature was kept constant at 27 °C during the experiment.

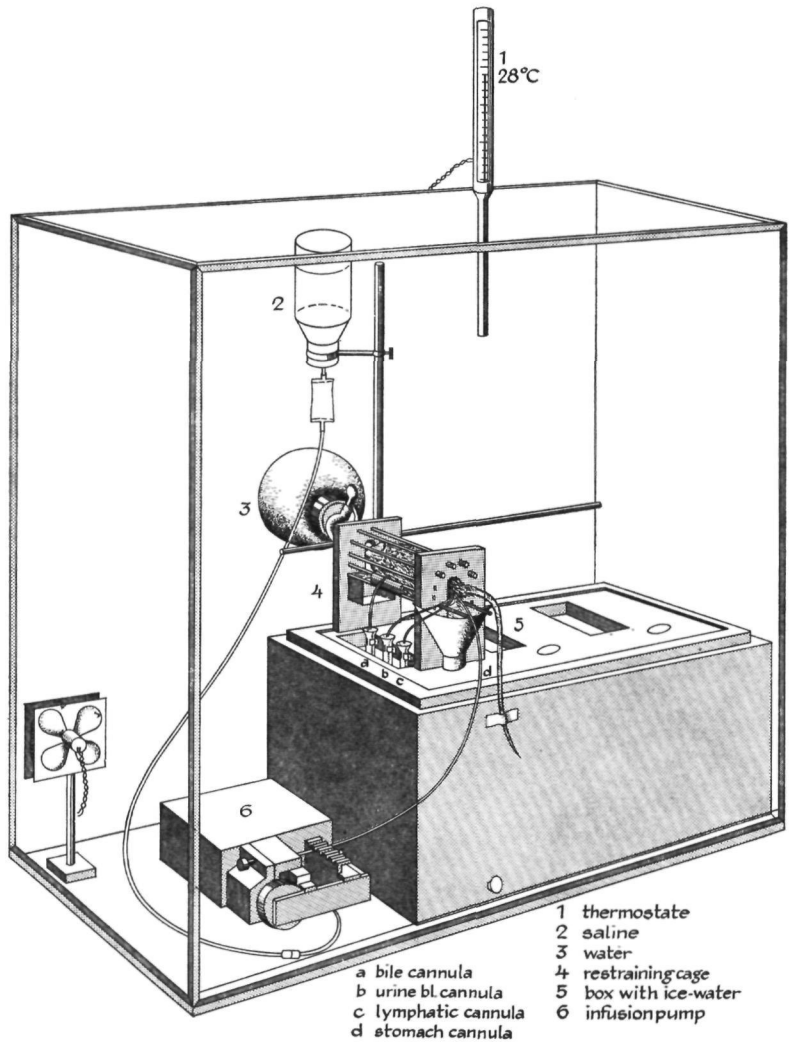


Fig. 2
Outline of the experimental set-up.

2.3.2.
Two kinds of experiments were carried out.

A. In the first series of experiments cannulae were placed in the thoracic duct, stomach and in the urinary bladder. In certain experiments bile was collected from the bile fistula (see chapter 3). During the whole experiment the rats were starved and saline infusion was given via the stomach cannula. 15-20 hr after the operation the infusion was interrupted by the administration of the pharmacon, followed by 1 ml saline. After administration lymph was sampled from 0-2, 2-4, 4-6, 6-8, 8-12 and 12-24 hr, urine and faeces and, in certain experiments bile, were sampled during the whole 24 hr period. After that period samples of cardiac blood and perirenal fat were taken under ether anesthesia. The liver and the intestine with stomach together with the intestinal contents were removed. A diagram of the experimental set up is given in fig. 3 A.

B. In the second series of experiments cannulations of the mesenteric duct, stomach and the portal vein were carried out. Before the operation the rats were starved for 15-20 hr. As described (this chapter 2.2.2.5.) heparin-saline infusion was given via the portal cannula. An infusion of saline via the stomach cannula was also conducted. In these experiments the pharmaca, followed by 1 ml saline, were administered directly after the operation via the stomach cannula. Lymph and blood samples were obtained ½, 1, 2, 3 and 4 hours after administration. The sample collection lasted from about 5 min. before until about 5 min after the times mentioned. The lymph which was collected from 3 hours 5 min until 3 hours 55 min after administration was used for the analytical determination of the unchanged pharmacon. For this determination in portal blood a sample, taken under ether narcosis, was used from about 4 hr after administration (see fig. 3 B).

2.4. STIMULATION OF THE LYMPH FLOW

Several investigators have shown that the thoracic duct lymph output rises when saline is given via the stomach (Yoffey and Courtice, 1970; Gallo-Torres and Neal Miller, 1969). Such an infusion is given since in the absence of infusion of saline into the stomach there is some lymph flow but it is minimal. If insufficient fluid intake is prolonged there is

usually obstruction of the catheter due to clotting of the lymph with subsequent loss of the preparation.
In the present experimental set up the rate of saline infusion was 2.5 ml/hr and an average of 1.5-2 ml/hr lymph was sampled.

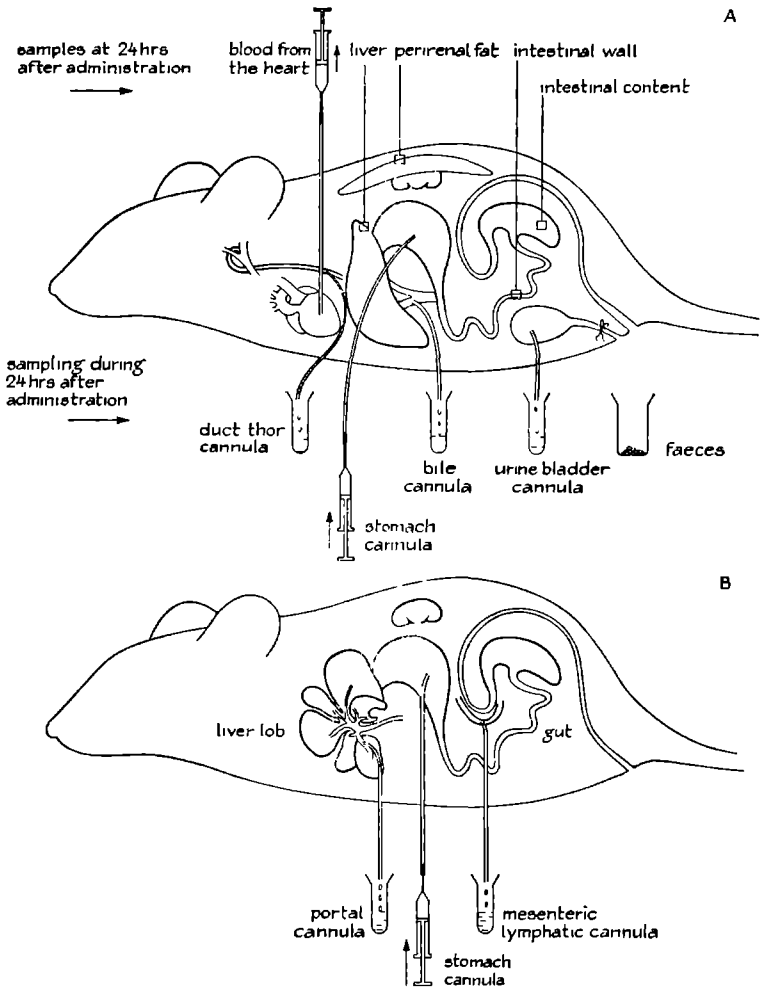


Fig. 3 A and B.
Fig. A shows the schematic picture of the experimental set-up with cannula construction and sampling system for experiment A
Fig. B shows the same for experiment B

2.5. PREVENTION OF CLOTTING

In order to prevent clotting of the lymph about 3 µl heparin (thromboliquine, 1 ml = 5000 i.u.) was introduced in the collecting vessel. This was also done to prevent clotting of blood. In those cases in which we wanted to study the chylomicrons and lipoproteins of the lymph we used EDTA (1 mg/ml) instead of heparin to prevent clotting. Moreover physical and chemical changes in the isolated chylomicrons and lipoproteins can be avoided by the addition of EDTA (Ontko, 1970).

2.6. MATERIALS

2.6.1. Unlabelled compounds

The following compounds were used during the investigations. DDD and DDE (Aldrich); aldrin and dieldrin (Analabs); n-butylphthalate, phthalic acid di- (2 ethyl-hexyl) ester = DOP, 3-methylcholanthrene (Eastman Kodak Co.); DDT (Fluka A. G. - Buchs S. G.); cyclandelate (Gist-Brocades); bishydroxycoumarine (S. A. F. Hoffmann-La Roche & Co Ltd.); butylated hydroxytoluene = B.H.T. (I.C.I.); chlorotrianisene (K & K Laboratories, inc.); cholesterol (Koch-Light Lab. Ltd.); lindane (Interpharm); cortisone, digitoxin, p-dimethylaminoazobenzene, phthalic acid, phthalic anhydrid, vitamin A, vitamin K_1 (Merck A. G.); ethynylestradiol, lynestrenol, β-sitosterol, vitamin E (Organon); 25-hydroxycholecalciferol = 25-HCC, vitamin D_3 (Philips Duphar); cholesterol palmitate, diethylstilbestrol, mestranol, 17-methyltestosterone, nandrolone, progesterone, testosterone (Sigma Chem. Comp.); quinestrol (Warner-Lambert Research Institute); 17-methyltestosterone-3-cyclopentyl-enol-ether, quingestrone (Warner-Vister); aroclor, 2,2′, 4,4′, 5,5′, hexachlorbiphenyl = H.B.F. (T.N.O. Zeist); Δ 1-tetrahydrocannabinol = Δ 1-THC (gift).
With the aid of thin layer chromatography impurities or by-products of the compounds above could not be detected. Investigated was also hexadecane (Baker). Identity and purity of this compound was examined by means of the boiling point. As constituents for the dosage forms the following compounds were used: lecithin 95-100 per cent (B.D.H.); sodium glycochenodeoxycholate, sodium glycocholate, sodium taurochenodeoxycholate (Calbiochem); glucose (N.V. Corn Products Comp.); sodium chloride (Merk A. G.); protifar* (Nutricia N.V.); mono-olein, oleic acid and triolein (Unilever Emery).

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* Trade name for a complex mixture of proteins

2.6.2. Radioactive compounds

A survey of the radioactive compounds used in the present investigations is given in table 1. All the compounds were tested with thin layer chromatography. The compounds were at least 98 per cent pure.

Table 1

Compound	Specific activity	Manufactured by
Aldrin-^{14}C(u)	52 0 mCi/mM	TRC Amersham
bishydroxycoumarin-		
-methylene-^{14}C	83 µCi/mg	Philips Duphar
cholesterol-4-^{14}C	50 mCi/mM	TRC Amersham
cyclandelate-carbonyl-^{14}C	1 1 µCi/mg	Gist-Brocades
DDD (p-chlorophenyl-^{14}C)		
= 1 1-dichloro-2 2-bis		
(p-chlorophenyl) ethane	1 11 µCi/mg	synthesized from DDT-^{14}C see 2 6 3
DDE (p-chlorophenyl-^{14}C)		
= 1 1-dichloro-2 2-bis		
(p-chlorophenyl) ethylene	1 11 µCi/mg	idem
DDT (p-chlorophenyl-^{14}C)		
= 1 1-bis-(p-chlorophenyl-^{14}C)		
-2 2 2 trichloroethane	23 9 mCi/mM	Fluka A G -Buchs S G
dieldrin-^{14}C	64 2 mCi/mM	TRC Amersham
diethylstilbestrol-		
-monoethyl-1-^{14}C	39 mCi/mM	TRC Amersham
p-dimethylaminoazobenzene-^{14}C	1 72 mCi/mM	N E N
D O P -carbonyl-^{14}C		
= phthalic acid di-(2 ethylhexyl) ester	1 3 µCi/mg	synthesized from phthalic anhydrid-carbonyl-^{14}C
hexadecane-1-^{14}C	47 2 mCi/mM	TRC Amersham
lindane-^{14}C (u)	54 mCi/mM	TRC Amersham
lynestrenol-4-^{14}C	10 1 µCi/mg	Organon
mestranol-4-^{14}C	145 uCi/mg	Organon
3-methylcholanthrene-6-^{14}C	5 47 mCi/mM	N E N
nandrolone-4-^{14}C	102 3 µCi/mg	Organon
phthalic anhydrid-carbonyl-^{14}C	8 19 mCi/mM	Mallinckrodt
progesterone-4-^{14}C	57 3 mCi/mM	N E N
quinestrol-6,7-^{3}H	572 µCi/mg	Warner Lambert Research Institute
Δ 1-THC-^{3}H (G)		
= Δ 1-tetrahydrocannabinol	142 µCi/mg	Δ 1-THC was tritiated by TRC Amersham The crude product was purified see 2 6 3
vitamin D_3-4-^{14}C	55 4 µCi/mg	Philips Duphar

2 6 3 Preparation of radioactive compounds

Certain compounds had to be synthesized or purified in our laboratory

DDD (p-chlorophenyl-^{14}C)

This was synthesized from DDT-^{14}C according to Langer (1944) To DDT (p-chlorophenyl-^{14}C), 23 9 mCi/mM, was added unlabelled DDT resulting in 75 mg DDT with a specific activity of 1 μCi/mg Mixing with 0 9 ml alcohol 96 per cent, 56 mg granulated zinc and 150 ml conc HCl followed This mixture was fluxed during 8 hr and thereafter extracted with chloroform The chloroform was evaporated and the remaining material purified on a pre-coated silica-gel glass plate of 2 mm thickness (Merck A G Darmstadt, Germany) Using the method of Abbott et al (1964) the plate was developed with n-hexane DDD was separated in this way from DDT and DDE After localization of DDD by scanning, silica-gel containing the adsorbed compound was scraped off the plate and eluted with n-hexane The yield was 40 per cent Identity and chemical purity were studied with a L K B gaschromatograph-Mass spectrometer, type 9000 of Bromma, Sweden, with column content OV-17 3 per cent, t° 180-220 The synthetized DDD proved to be more than 98 per cent pure The specific activity of the DDD was 1 11 μCi/mg

DDE (p-chlorophenyl-^{14}C)

For the synthesis of DDE the principle (Bailey et al, 1969) that DDT splits off HCl easily in alcoholic KOH was used DDT-^{14}C, 23 9 mCi/mM, was mixed with unlabelled DDT resulting in 50 mg with a specific activity of 1 μCi/mg This product was mixed with 2 5 ml 10 per cent alcoholic KOH, and shaken during 1 hr After that an extraction with petroleum benzin (b p 40-60 °C) was carried out The petroleum benzin was evaporated and the remaining material purified on a silica-gel glass plate as described for DDD Identity and chemical purity were also tested in the same way DDE proved to be more than 98 per cent pure A yield of about 70 per cent was obtained The specific activity of the synthesized product is 1 11 μCi/mg

D O P -carbonyl-^{14}C

This compound was synthesized from phthalic anhydride-carbonyl-^{14}C according to Iwakura (1945) Phthalic anhydride-carbonyl-^{14}C, 8 19 mCi/mM, was mixed with unlabelled phthalic anhydride resulting in 29 6 mg, that is 0 2 mM, with specific activity 100 μCi/0 2 mM This

product was mixed with 1.5 ml dry benzene, 0.5 mM 2-ethyl-hexanol (65 mg = 78 µl) and a small drop (added with capillary) of H_2SO_4. This mixture was refluxed overnight and thereafter the benzene was boiled off. The remaining material was dissolved in ether and purified according to Braun (1965) on a pre-coated silica-gel glass plate of 2 mm thickness. The plate was developed with methylene chloride. Silica-gel containing the adsorbed product was scraped off the plate and eluted with ether. A yield of about 95 per cent was obtained. The synthesized compound had a specific activity of 1.3 µCi/mg. The identity and chemical purity were tested as described for DDD. The compound proved to be more than 98 per cent pure.

Δ 1-Tetrahydrocannabinol-^{3}H (G).
The unlabelled product was received as a gift. Part of it was tritiated by TRC Amersham. The tritiated crude product was purified on a pre-coated silica-gel glass plate G of 2 mm thickness. The plate was developed with benzene. After localization, silica-gel with the adsorbed compound was scraped off the plate and eluted with ether. Identity and chemical purity were tested with the L.K.B. gaschromatograph-mass spectrometer as described for DDD-^{14}C. The product with a specific activity of 142 µCi/mg proved to be pure.

2.7. ANALYTICAL PROCEDURES

2.7.1. Sampling procedures.
Radioactivity in lymph-, urine- and tissue samples was determined in the usual way.

2.7.1.1. Lymph.
A. In the first series of experiments (see 2.3.2.A.) lymph was sampled in a volumetric flask of 25 ml, the amount was determined by weighing and thereafter this lymph was diluted with saline to 25 ml. Two portions of 4 ml of the diluted lymph were pipetted into two liquid scintillation counting vials and the radioactivity was determined (see 2.7.2.1.).

B. In the second series of experiments (see 2.3.2.B.) the lymph that had been sampled at ½, 1, 2, 3 and 4 hr after administration was put into two counting vials, the amount (0.1 g) measured by weighing and the radioactivity determined. The lymph, produced 3 hr 5 min - 3 hr 55 min after administration was sampled and the amount measured by

weighing. A portion (0.1 g) was put into a counting vial and the radioactivity determined. This was done in duplicate. In the rest of this lymph (1 g) the drug together with lipoids were extracted according to the procedure of Bligh and Dyer (see 2.7.3.). In this extract the amount of unmetabolized product was determined by means of reversed phase chromatography (see 2.7.4. and 4.2.3.). Since the total amount of radioactivity and the amount of unchanged drug in the lymph sample can be estimated, the percentage unchanged drug can also be calculated (see 2.7.4.).

2.7.1.2. Cardiac blood and portal blood.
Cardiac blood in the first series of experiments (see 2.3.2.A.) was sampled at once into two counting vials and the amount (0.2 g) measured by weighing. Radioactivity was determined according to 2.7.2.1. Portal blood, sampled (0.2 g) in the second series of experiments (see 2.3.2.B.) at ½, 1, 2, 3 and 4 hr after administration of the drug, was treated similarly to cardiac blood. In a certain number of experiments (see 2.3.2.B. and chapter 4.2.1.) at 4 hr after administration of the drug 1.4 g of portal blood was taken under ether anesthesia. Two parts (0.2 g) of this sample were treated similarly to cardiac blood. The rest (1 g) of these samples was diluted with aqua dest. to 4 ml and extracted. The percentage unchanged drug in these blood samples was determined in the same way as for the lymph samples mentioned under 2.7.1.1.B.

2.7.1.3. Intestine and stomach; faeces and intestinal contents.
A. Intestine.
The intestine with contents was weighed. Thereafter the intestinal contents were rinsed out of the intestine with 5 changes of saline, finally the intestine was emptied and, after drying the outside between filter paper, weighed. The weight of the intestinal contents was obtained by subtracting the above values. The intestine, made up to a weight of 25 g with aq.dest. was homogenized in a waring blendor for 2 min. The radioactivity was determined in two portions of 4 g of the homogenate (see 2.7.2.1.).

B. Faeces and intestinal contents.
The diluted intestinal contents (see A.) were mixed with the weighed faeces and aq.dest. to a weight of 50 g. Homogenisation in a waring blendor for 2 min followed. The radioactivity was determined in two portions of 2 g of the homogenate (see 2.7.2.1.).

2.7.1.4. Urine.
Urine was sampled in a volumetric flask of 50 ml. The sample was made up to 50 ml with aq.dest. Two portions of 10 ml were placed in two counting vials and the radioactivity was determined (see 2.7.2.1.).

2.7.1.5. Bile.
Bile was treated similarly to the lymph of the first series of experiments (2.7.1.1.A.).

2.7.1.6. Liver.
The liver was weighed, thereafter water was added to a weight of 30 g and the whole was homogenized in a waring blendor. In two portions of 2 g of the homogenate the radioactivity was determined as decribed for blood (see 2.7.1.2.).

2.7.1.7. Perirenal fat.
Perirenal fat was weighed and a mixture of aq.dest. - isopropyl alc. 1 : 1 was added to a weight of 15 g and the whole was homogenized for 2 min in a waring blendor. In two portions of 2 g of the homogenate radioactivity was determined according to 2.7.2.1.

2.7.2. ASSAY OF ^{14}C- AND ^{3}H-RADIOACTIVITY

2.7.2.1. Liquid scintillation counting (LSC).
Radioactivity in all animal samples, blood- and liver samples excepted, was determined by mixing with 10 ml instagel (Packard) in a liquid scintillation counting vial followed by counting the radioactivity. This was performed in a Packard Tri-Carb liquid scintillation spectrometer model no 3380, equiped with an Absolute Activity Analyzer, model no 544 (Triple A). Blood samples (0.2 g) and liver homogenates (2 g) were treated in a different way. The sample was mixed in a counting vial with 1.5 ml soluene - 100 (Packard) - isopropanol 1 : 1 and the mixture warmed for 15 min at 40 °C in a waterbath with shaking. Then 0.5 ml H_2O_2 (35 per cent) was added with shaking and finally after 30 min 15 ml of 0.5 N HCl-instagel 1 : 9 was added to it and the radioactivity was counted.
Counting of ^{14}C radioactivity.
An AES-quench curve was made with the aid of ^{14}C-hexadecane, quenched with different amounts of chloroform. The triple A was adjusted by means of the counting efficiencies of this quench curve.

Counting efficiencies were as a rule higher than 65 per cent. Below this percentage corrections of measured CPM to DPM were calculated with the aid of the internal standard method.
Counting of ^{3}H-radioactivity.
An AES-quench curve was made in the same way as for ^{14}C, but using ^{3}H-hexadecane. Since tritium has a much lower counting efficiency than ^{14}C, corrections of measured CPM to DPM for every determination were made by means of this quench curve. When the counting efficiency was under 15 per cent CPM corrections were made with the aid of the internal standard method. The yield of ^{14}C- and ^{3}H- radioactivity was checked in all tissue samples investigated by us. A known amount (100,000 DPM) ^{14}C- and ^{3}H-hexadecane was mixed with the non-active samples and the radioactivity determined. The yields were greater than 99 per cent of the added amount.

2.7.2.2. Scanning of the thin layer chromatograms.
When radioactive spots on thin layer chromatography (TLC) plates were detected with a scanner, a Berthold Dünnschicht-scanner II was used. This scanner was equiped with a synchronously recording Metrawatt A.G. writer.

2.7.3. EXTRACTION OF LIPOPHILIC PRODUCTS

For the extraction of fat soluble compounds the procedure of Bligh and Dyer was used (1959). This method is generally employed for the extraction of lipophilic compounds by Avioli et al (1967). The lipoid extractions were performed in series.
A quantity of 4 ml or 4 g of tissue sample was homogenized and shaken for 10 min with a mixture of 5 ml chloroform and 10 ml methanol. The resulting homogeneous mixture was sucked off via a glass scintered filter, covered with celite 545. The celite filter was washed with 10 ml of chloroform and the combined filtrates shaken for 10 min. Thereafter 5 ml of water added and shaken again for 10 min. After separation the lower layer contains the compound (called layer A.). In order to obtain a quantitative extraction the upper layer was again treated with 10 ml of chloroform, added by sucking off via the celite filter. The chloroform layer, obtained after separation was mixed with chloroform layer A. The resulting solution contained the compounds to be measured. This fraction was concentrated under a flow of N_2.

The method described was checked with ^{14}C-cholesterol and ^{14}C-DDT. A recovery of at least 99 per cent was achieved. If separation did not occur the sample was centrifuged for 5 min at 3000 r.p.m.

2.7.4. THIN LAYER CHROMATOGRAPHY

2.7.4.1. Reversed phase chromatography.

In particular reversed phase chromatography on silica-gel plates, coated with silicon oil, was performed according to Biagi et al (1969). Pre-coated silica-gel glass plates, 20 x 20 cm, with and without fluorescence indicator (F 254) (Merck A.G.) were used. The plates were activated at 120 °C for half an hour and cooled in a dessicator with dry silica-gel. They were then impregnated in a chromatographic chamber, containing 200 ml silicon solution. The silicon solution consisted of 5 per cent by weight of silicone MS 200 (350 CS) (Midland Laboratories). Acetone/aq.dest 60/40 v/v was used as the solvent system. An exception was made for the determinations of the ΔR_m values (see 4.2.4.). In the latter case the ratios from v/v 30/70 - 85/15 were used.

The plates were developed at room temperature over about 15 cm. Spots were detected by: 1. Scanning of radioactivity. 2. U.V. absorption of spots applied on plates with fluorescence indicator. 3. Spraying with a solution of $KMnO_4$. This solution was freshly prepared before spraying by mixing: 1 per cent $KMnO_4$ in aq.dest. with an equal amount of 5 per cent Na_2CO_3 in aq.dest. After spraying, the plates were heated for some minutes at 120 °C. The compounds were detected as yellow spots on a violet background. Since lipophilic drugs were investigated this method had the advantage of being applicable to all of them with significantly different R_f-values.

2.7.4.2. Determination of radiochemical purity.

Two portions of radioactive drug (100,000 DPM) were pipetted into counting vials and the amount (A) of radioactivity was determined (see 2.7.2.). The same amounts were developed on a reversed phase plate. After localization by scanning of the radioactive product, silica-gel with adsorbed compound was scraped off the plate and put in a counting flask. To this flask 2 ml aq.dest and 10 ml instagel were added. The amount of radioactivity (B) was then determined. The percentage of radioactive pure drug was calculated by the formula B/A x 100.

2.7.4.3. Determination of percentage unchanged pharmacon in lymph, blood and intestinal contents.
This was performed with the procedures described under 2.7.1.1.B. and 4.2.3. The extraction residue was handled according to the procedure mentioned for the determination of radiochemical purity (2.7.4.2.). After localization by scanning the developed area was divided into 15 bands. These bands were scraped off and put into counting flasks. The radioactivity was determined in the same way as described under 2.7.4.2.

2.7.4.4. Determination of percentages of DDT and aldrin and their respective metabolites in perirenal fat.
These determinations were performed as described in 2.7.1.7. and 2.7.4.3. except that the extraction residue, before developing on a reversed phase plate, was purified by means of an aluminium oxide column (see 6.2.2.).

CHAPTER 3

FACTORS INFLUENCING THE ABSORPTION OF DDT VIA LYMPHATIC AND PORTAL SYSTEMS

3.1. INTRODUCTION

In order to determine whether the administration form influences the route of drug absorption either via the portal system or via the lymph tract, investigations were carried out with DDT in several application forms. Much attention has been paid to the fate of this compound in the body. Because it is highly lipoid soluble, it accumulates in the fat tissues. It has been shown previously that DDT, given in a fatty dosage form, is absorbed in considerable amounts via the lymph tract (Rothe et al, 1957; Cohn and Sieber, 1970). So DDT is of importance, not only as an accumulating agent, but also as model compound for studying the factors which influence absorption.
In the present study the following dosage forms were used.

1. **Propylene glycol** was employed as the non-fatty dosage form. In fact DDT is insoluble in water. So another application form different from fat but physiologically inert and miscible with water had to be chosen. Propylene glycol is widely used as a non-toxic drug solvent (Zaroslinski et al, 1971), and DDT can be dissolved in it.

2. **Triolein** was chosen as the fatty vehicle for DDT. It has been shown for cholesterol that good rates of lymph transport are reached by feeding the sterol dissolved in triolein (Sylvèn and Borgström, 1968). It appeared also that DDT can easily be dissolved is this olein. The triolein was prefered to the more commonly used vegetable oils (like sesam oil) because the latter are not homogeneous but consist of a mixture of several components. The experiments with triolein were used on rats with and without bile cannulation.

3. As vehicle of administration in another series of experiments a so

called **micellar solution** was prepared. In the physiological situation fat components of the food are transformed to a micellar solution by the juices of the gut, before absorption from the lumen of the gut. The micellar solution is very important for a good absorption of these nutrients. For this reason Gallo-Torres developed a micellar solution containing components like conjugated bile salts, lecithin, protein etc. to fascilitate maximal absorption of lipoids like vitamin E and cholesterol (Gallo-Torres et al, 1969; Gallo-Torres, 1970). The composition of our micellar solution was chosen in such a way that it fullfilled as well as possible two purposes:

x. The composition is like that of fats just before absorption by the mucosa, in other words is like a natural micellar gut solution (Treadwell and Vahouny, 1968; Dobbins, 1969).

x. The components are present in amounts similar to those in chylomicrons (see chapter 5.1.).

Also in these experiments the possible influence of bile was investigated by means of cannulation. In addition bile cannulation after dosing of micellar solution gives information about entero-hepatic circulation of DDT. The comparison of the absorption of DDT via the different dosage forms may give us more information about the following question: What must be the properties of the administration form in order to give maximal absorption of DDT via the lymph tract? An extrapolation with respect to other drugs could be applied and may have practical value.

3.2. MATERIALS AND METHODS

Cannulation and sampling was carried out as described in chapter 2 (2.3.2.A.). p,p' – DDT (phenyl-^{14}C) was mixed with unlabelled DDT to a specific activity of 1 μCi/mg. At the start of the experiment each animal received 1 mg DDT. As will be shown in chapter 4, in lymph sampled from 3-4 hr after administration of DDT (the peak of DDT absorption in lymph) 97 per cent of the radioactivity is represented by unchanged p,p' – DDT. On this basis it may be assumed that, also during the experiments described here, the amount of metabolized DDT is negligible. The experimental conditions, in particular with respect to the dosage form are given in table 2.

Table 2. DDT administration under various experimental conditions.

exp	dosage form		bile flow*	number of rats
A	micellar sol	4 ml	—	5
B	triolein	0 8 ml	—	5
C	propylene glycol	0 3 ml	+	5
D	triolein	0 3 ml	+	4
E	triolein	0.8 ml	+	6
F	micellar sol.	4 ml	+	5

* refers to bile cannulation, which prevents the bile flow.

Preparation of the micellar solution.
The composition of 4 ml micellar solution is as follows:

I.	mono olein	90	mg
	egg lecithin	32	mg
	cholesterol	15	mg
	oleic acid	163	mg
II.	sodium taurocholate	270	mg
	sodium taurochenodeoxycholate	10	mg
	sodium glycocholate	35	mg
	sodium glycochenodeoxycholate	3	mg
	sodium chloride	13.5	mg
	glucose	880	mg
	saline	2.65	ml
III.	amylopectin	120	mg
	protifar (protein)	32	mg

The solution is prepared as follows: the fat components give under I are weighed in a bottle under N_2. The flask is closed and the fat components are melted by placing the flask in boiling water. Of this fat mixture 300 mg for every portion of micellar solution is weighed in a tube already containing DDT-^{14}C. After dissolving the DDT, the components of II are weighed and added to the mixture. This is placed in ice and mixed with an ultrasonic disintegrator (M.S.E.) for 50 sec. (5 x 10 sec. with intervals of 1 min.). The pH of the solution is adjusted

with HCl to 6.5, the pH of the gut. The two components of III are added to the mixture and mixed with an Ultra-Turrax (Janke und Kunkel K.G.).
It was checked that at pH 1.7 (pH in the stomach) this micellar solution, diluted with saline 1 : 3, remained clear for 3 hr.

3.3. RESULTS

3.3.1. Scheme of absorption.

The absorption of DDT in the organism is schematized in fig. 4.
Orally taken DDT is absorbed for a certain part from the gut. Theoretically the absorption from the gut can be divided into transport via the lymph and via the portal system. A certain part of the amount absorbed via the portal system is excreted in the gut again via the bile. The following symbols are used for the various amounts of DDT present in the different body constituents:
L means the amount of DDT transported via the thoracic duct. This amount, measured after collecting during a certain period, is expressed as a percentage of the dose (D) administered.
B means the amount of DDT excreted in bile as a percentage of the administered dose. In certain cases the value for B was measured after cannulation. Otherwise an approximation was used.
G means the amount of DDT that remained in faeces, intestinal contents and the wall of the gut and stomach. In those experiments in which bile flow was not prevented by bile cannulation, the value was corrected for the relatively small amount of DDT, excreted via the bile (B) by subtraction.

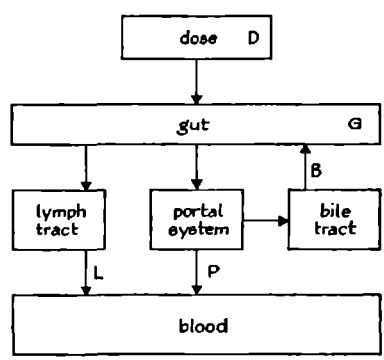


Fig. 4

P is the amount of DDT absorbed via the portal system diminished with the amount of DDT excreted in the bile. P, as a percentage of the administered dose was obtained by calculation. The value P can be derived with the aid of the following assumption: L + B + G + P = 100 per cent.

3.3.2. Excretion of DDT via bile.

For a good evaluation of the given scheme (fig. 4), it is of importance to know to what extent DDT is excreted via the bile. Up to now little attention has been paid to this phenomenon. Two different kinds of experiments were carried out. In the first series of experiments DDT was administered in a micellar solution, containing different bile components. In the second series the drug was given in olein preparation. At 24 hr after administration the amount of DDT present in the bile, collected via cannulation, was determined and this value was compared with the amounts present in lymph, portal system and the gut. The results are presented in fig. 5. It appeared that in the case of administration in a micellar solution about 3 per cent of the dose administered was excreted via the bile. This amounts to 5.9 per cent of the DDT, absorbed via the portal system: B/(P + B). In the second series of experiments a quite other pattern was observed. It appears that DDT

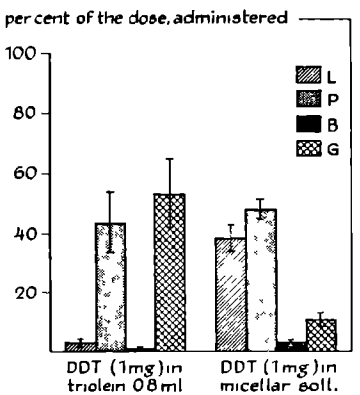


Fig 5

Transport of DDT via different routes in bile - cannulated rats. The amounts of DDT are presented as percentages of the doses administered. Means and standard deviations are given for 5 separate experiments. L, B and G are the amounts of DDT, measured resp in lymph, bile and gut P is the amount of DDT absorbed via the portal system, calculated for each experiment as follows: P = 100 per cent - (L + B + G). Samples were obtained during or at 24 hr after administration

transport via the lymph tract is very low, but a considerable amount is absorbed via the portal system. Nevertheless practically no secretion with the bile occured. Presumably this is due to a decreased production of bile fluid. It can also be observed that in the latter case a great amount of DDT was not absorbed from the gut.

3.3.3. Comparison of the different dosage forms.

The amounts of DDT absorbed via the different routes in 24 hr dependent on the different dosage forms are summarized in fig. 6. As far as the excretion via the bile is concerned, the values given are based on an approximation. From the experiment described above (3.3.2.), in which DDT was administered in a micellar solution – a good imitation of the physiological situation – it was found, that B/(P + B) was 5.9 per cent. It was assumed that this ratio also hold true in the present cases, in which no bile cannulation was done. So for the experiments presented in fig. 6, the values of B were derived after calculation of P. It was assumed, that no reabsorption of DDT, excreted with bile, occured.

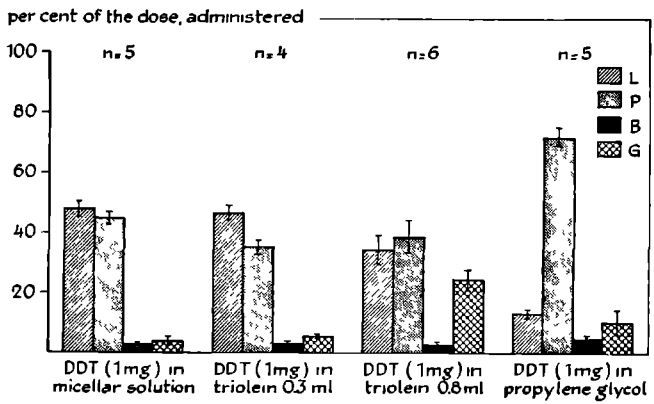


Fig. 6

Influence of different dosage forms on the transport of DDT via different routes. The amounts of DDT are presented as percentages of the doses administered. Means and standard deviations are given; n refers to the number of experiments. L is the amount of DDT, measured in lymph. G is the measured amount of DDT in the gut, corrected for the small amount of DDT excreted via the bile. P is the amount of DDT absorbed via the portal system, calculated for each experiment as follows: P = 100 per cent - (L + G measured). B is an approximated value of the amount of DDT transported via the bile. Samples were obtained during or at 24 hr after administration.

As can be seen in fig. 6, if DDT is administered in a non-fatty solution like propylene glycol, considerably less absorption via the lymphatics takes place as compared to the DDT administration in triolein 0.3 ml, triolein 0.8 ml and in micellar solution (resp. $P < 0.001$, $P = 0.002$, $P < 0.001$; F-test).
However, if a solution of propylene glycol is used, DDT to a greater extent is absorbed via the portal system as compared to triolein 0.3 ml, triolein 0.8 ml and micellar solution (resp. $P = 0.002$, $P < 0.001$, $P = 0.001$; F-test). The amount of DDT, which is not absorbed, and therefore is left in the faeces and other intestinal constituents (G), is significantly greater for the group of rats which received DDT in triolein 0.8 ml as compared with the other groups, namely triolein 0.3 ml, micellar solution and propylene glycol (resp. $P = 0.004$, $P = 0.001$, $P = 0.02$; F-test). If triolein 0.3 ml and the micellar solution are considered as dosage forms no obvious differences among the amounts of DDT absorbed via the different routes are observed.

3.3.4. Distribution of DDT absorbed via the portal system.
In the experiments described up to now, done with lymph-cannulated rats, the amount of DDT absorbed via the portal system is deduced form measured values. If, indeed, an amount of ^{14}C-DDT is transported by the blood of the portal vein, then radioactivity must be found in the rest of the body and in the urine. Therefore in some rats the amount of radioactivity in urine during 24 hr and in blood, liver and perirenal fat after 24 hr was investigated. In the experiments the dosage form with micellar solution and with propylene glycol was employed (see table 3). It appears, that for the micellar solution, as well as for the propylene glycol administration, about 0.15-0.20 per cent is present in the blood. In the liver the amounts are about 10 times higher. Only a small percentage of the absorbed amount is excreted in the urine (< 1 per cent).
It is obvious that a major part accumulates in body fat. In 1 g of perirenal fat 1.7 and 1.6 per cent is found, after administration in micellar solution and resp. 2.6 and 2.7 per cent after administration of ^{14}C-DDT in propylene glycol.

According to the experiments of Reed et al (1930) the total amount of fat in the rat can be approached. On the basis of their data 22 g of fat can be expected in the rats used by us. It was assumed that the radioactive products are present in total body fat in the same ratio as in perirenal fat. Thus the amount in total body fat can be derived

Table 3 Distribution of radioactivity* in lymph-cannulated rats after ^{14}C-DDT administration and with calculated absorption by portal blood.

Vehicle of transport	Micellar solution	Micellar solution	Propylene glycol	Propylene glycol
total blood**	0 15	0 15	0 15	0 20
total liver	1 3	1 4	2 2	1 5
total body fat***	37 4	35 2	57 2	59 4
total urine	0 60	0 70	0 15	0 45
sum	39 45	37 45	59 70	61 55
calculated absorption by portal blood****	50 8	58 2	76 2	70 0

* ^{14}C-radioactivity, sampled during or after 24 hr, is given as percentage of the administered dose

** total blood is estimated as 1/15 of body weight

*** the amount of radioactivity in total body fat is estimated from the amount in 1 g perirenal fat

**** for the calculation of these values see ch 3 3 1

from the amount measured in 1 g of perirenal fat. In this way it can be calculated that 40-60 per cent product is present in the total body fat. As can be seen in table 3 radioactivity was not determined in all parts of the body of the rat. Yet, when the given amounts are added and compared with the calculated absorption by portal blood, only 10-20 per cent of the portal absorption cannot be accounted for. After administration of DDT in propylene glycol the calculated absorption by portal blood is about 20 per cent more than after administration in micellar solution. This greater amount must be distributed in the body and indeed, as can be seen in table 3, the amount of product stored in body fat after administration in propylene glycol is about 20 per cent greater than after administration in micellar solution.

3.3.5 Lymphatic absorption of DDT as a function of time.

In the absorption process of DDT from the intestinal contents to the lacteals or portal system two steps can be distinguished:

1. Absorption of DDT from the intestinal contents to the inside of the mucosa cells. 2. Transport of DDT from the mucosa cells to the lacteals or the portal system. In principle the dosage form can influence both steps. An important question, posed in the introduction (3.1) is to

determine whether the dosage form influences the route of drug absorption either via the portal system or via the lymph tract. This question in fact concerns step 2. namely transport of DDT from the mucosa cells to lacteals or portal system. Therefore, in order to get an adequate comparison, the results of the lymphatic absorptions related to time, are presented as percentages of the total amount of DDT absorbed during 24 hr from the gut (Lt/L + P + B). The pattern of lymphatic DDT absorption was studied during 24 hr (see fig. 7).

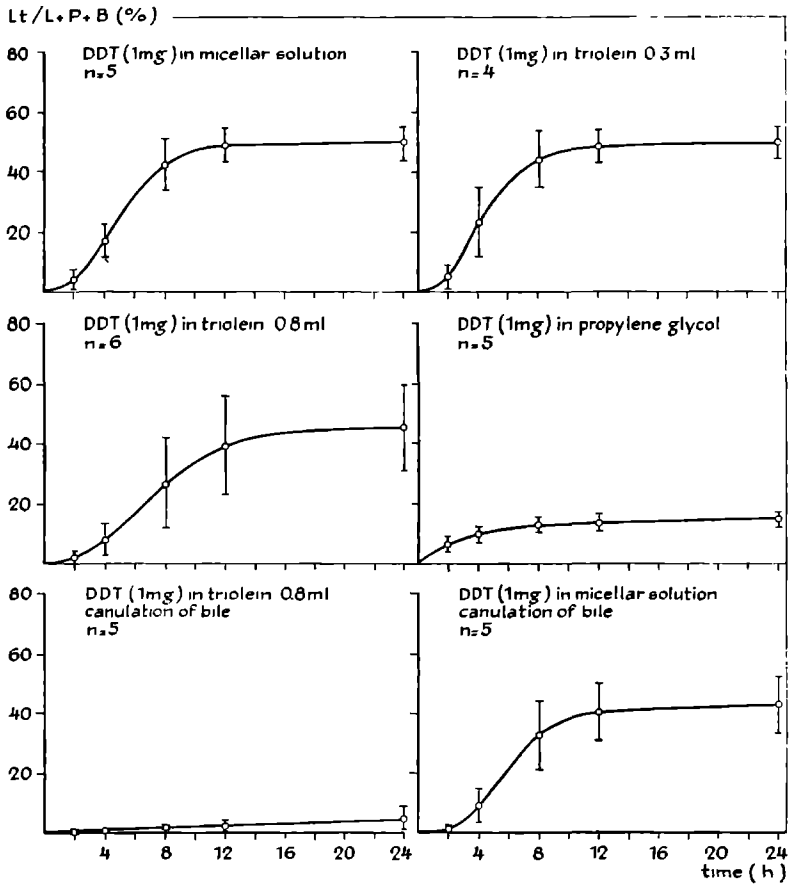


Fig. 7
Influence of dosage forms and bile cannulation on cumulative lymphatic absorption of DDT. The amount of DDT, absorbed via the lymph tract (L_t), is presented as a percentage of the total amount (L + P + B) absorbed during 24 hr. Means and standard deviations are given n refers to the number of experiments

The amount of DDT absorbed via the lymph tract (Lt) was determined from 0-2, 0-4, 0-8, 0-12 and 0-24 hr after administration

3 3 5 1 Comparison of different dosage forms
As is shown in fig 7, the curves of cumulative lymphatic DDT absorption, obtained after dosing of DDT in micellar solution or in 0 3 ml triolein are practically equal However after administration of DDT in propylene glycol the lymphatic absorption occurs more slowly and the total amount absorbed during 24 hr is lower For all dosage forms it can be observed that the absorption is almost completed within 12-24 hr So 24 hr sampling of lymph seems suitable to obtain a good measure for total lymph absorption

3 3 5 2 Influence of bile cannulation
Administration of DDT in micellar solution to bile-cannulated rats results in a pattern of lymphatic absorption that is fairly comparable to the pattern after administration of micellar solution whithout bile cannulation This means, that the micellar solution used is a good substitute for natural bile and a good imitation of the physiological situation is reached In contrast, when DDT is administered in 0 8 ml triolein to bile-cannulated rats the lymphatic absorption is very low, namelijk 5 1 $\pm$ 3 8 (s d) per cent This means that about 95 per cent of the total amount absorbed is transported via the portal system

3 4 DISCUSSION

In these investigations several aspects of the absorption of DDT from the gut can be clearly recognized As to the question whether the administration form influences the route of drug absorption either via the portal system or via the lymph tract, the answer is in the affirmative As is mentioned in the results (see 3 3 3 and fig 6) if DDT is administered in the non-fatty solution propylene glycol, considerably less absorption via the lymphatics takes place as compared to DDT administered in the fatty dosage forms like triolein and micellar solution However, DDT administered in propylene glycol is to a greater extent absorbed via the portal system than DDT administered in the mentioned fatty dosage forms So it is also clear that DDT, which is highly lipoid soluble, is not only absorbed well from a fatty administration form, but also from a non-fatty administration form, be it via an alternative route

From additional experiments in which DDT was administered in a non-fatty dosage form like tragacanth it appeared that the apparent lymphatic absorption was also low (7.2 per cent). Further it was observed, that in these cases the amount of DDT, which was left unabsorbed in the faeces and other intestinal contents was relatively high (61 per cent) as compared to DDT administered in propylene glycol.
The finding, that DDT administered in triolein to rats that are bile-cannulated, is still absorbed for about 47 per cent, mostly via the portal blood (see 3.3.2. and fig. 5) is remarkable. This finding may be interpreted with the results of the experiments of Gallagher et al (1965). They observed that 0.1 ml of radioactive triolein administered to rats with a bile fistula, was absorbed for about 60 per cent. Therefore the mentioned absorption of DDT might have occurred together with triolein. When DDT is administered in micellar solution to rats that are bile-cannulated not only is the portal absorption considerable, but the lymphatic transport is also considerable. Only about 10 per cent is left unabsorbed in the gut. It might be concluded that besides fat components, bile seems to be particularly necessary for lymphatic transport of DDT. As to the question which of the dosage forms, 0.3 ml triolein or 0.8 ml triolein or micellar solution is best used to give a maximal lymphatic absorption of DDT, the answer is as follows: Not only the amount of DDT left unabsorbed in the gut is significantly greater for DDT, administered in 0.8 ml triolein, but also the pattern of lymphatic absorption seems slower and more irregular. Therefore, in order to reach maximal lymphatic absorption, the dosage forms 0.3 ml triolein and micellar solution have to be used. No difference as to the absorption pattern of DDT is seen between these two dosage forms, when bile production is normal.
From the experiments with bile cannulation it might be concluded that the micellar solution is preferable in those cases in which normal bile flow is absent or disturbed.
Summarizing it might be stated, that the dosage form can influence the route of drug absorption, either via the portal system or via the lymph tract. Micellar solution or 0.3 ml triolein can be used equally well as dosage form to obtain an optimal lymphatic absorption of DDT.

CHAPTER 4

QUANTITATIVE CORRELATION BETWEEN LYMPHATIC ABSORPTION AND FAT SOLUBILITY

4.1. INTRODUCTION

As mentioned before, considerable transport of certain pharmaca via the lymph tract is known, following oral administration of these pharmaca in a fatty vehicle. It will be necessary to test more pharmaca in order to obtain a clear picture of the principles governing this considerable lymph transport. The pharmaca in respect of which this large lymph transport is known are strongly fat soluble. On the other hand it has been shown for a number of non – fat soluble medicines that they, following oral administration, scarcely penetrate the lymph tract at all (Sieber, 1970). Based on these empirical observations the examination of the degree to which lymph transport can be correlated with the measure of fat solubility of a pharmacon is obvious.
As a measure for the fat solubility of the investigated pharmaca the ΔR_m value in respect of butter yellow was dertermined. This term, is related to the partition coefficient, i.e. the distribution of the pharmacon between fat or a fat solvent on one side and water on the other, which is generally used as a measure for the lipophilicity of a compound (see 4.2.5.). As a measure of the lymph transport of the pharmaca studied, the transport in thoracic duct lymph during 24 hr following administration was taken. In order to obtain the best possible picture of the correlation described, the following points must be taken into account:

– As has already been discussed for the lymphatic absorption of DDT (see chapter 3.3.5.) two steps can be distinguished in the absorption process of the pharmacon from the intestinal contents to the lacteals or portal system: A. Absorption of the pharmacon from the intestinal contents to the inside of the mucosa cells. B. Transport of the pharmacon from the mucosa cells to the lacteals or the portal system. In priciple, both steps can be influenced by several factors. Therefore, to obtain the correlation between the ΔR_m value and the lymphatic absorption of the pharmacon as accurately as possible, the quantity

of pharmacon absorbed by the lymph during 24 hr as a percentage of the total quantity of pharmacon absorbed from the intestine was taken as a parameter for lymphatic absorption. The quantity of a pharmacon absorbed from the intestine cannot be exactly determined, because to a small extent a pharmacon, already absorbed from the gut, can be secreted back again in the gut with bile. Therefore the lymphatic absorption during 24 hr was expressed as a percentage of "the apparent absorbed activity".

- The question as to whether the lymphatic absorption is complete whithin 24 hr after administration of the pharmacon could be answered by collecting the lymph in several fractions. The pattern of the lymphatic absorption of the pharmacon during 24 hr could be observed in the curves of cumulative lymphatic absorption constructed from the analysis of these fractions.

- Either following oral administration or parenteral administration, pharmaca are always present to a certain extent in sampled thoracic duct lymph, even if these pharmaca are strongly soluble in water. In the introduction (chapter 1.1.A and 1.1.B) this phenomenon has already been remarked upon. Both the blood capillaries and lymphatic vessels appear to be freely permeable to small molecular weight compounds (Landis, 1962) and therefore, soon after administration, different sorts of pharmaca appear to be present in blood and lymph in nearly the same concentration (Levine et al, 1969). In view of the flow-ratio of portal blood and mesenteric lymph, which is approximately 500 (post absorptive for the rat) the absorption from the intestine will, under these circumstances, occur almost solely via the portal tract. Dependent on their pharmaco-kinetic pattern of distribution, metabolism and excretion, the pharmacon molecules will, following absorption via the portal blood, for some time be present in the circulation to a decreasing degree.
During this time the pharmacon molecules will interchange continuously between blood, interstitial tissue fluid and lymph. After the absorption from the intestine has been completed, pharmacon molecules from the circulation may appear in the thoracic duct lymph and be sampled with this. The percentage of the pharmacon absorbed out of the intestine which accumulates in the lymph during 24 hr will thus in general be related to pharmaco-kinetic parameters, such as the blood levels and half-life time of the pharmacon and to the flow of the thoracic duct lymph and not directly to the lipophilicity of the phar-

macon. In a number of investigated cases (Sieber, 1970) this percentage appears not to be constant, but to vary between 0.2-8 per cent of the quantity absorbed from the intestine. Therefore with a 24 hr absorption in the order of 0-8 per cent of the absorbed pharmacon it cannot, in general, be attributed to specific absorption via the lymph, although specific absorption via this lymph tract can be involved. If during the absorption the concentration of the pharmacon in mesenteric lymph appears to be many times greater than in portal blood, then this provides evidence for specific absorption via the lymph tract. To investigate this we have determined the quotient of the concentrations in lymph and portal blood during absorption.

- Practically all the pharmaca in our investigation were labelled with ^{14}C or ^{3}H. The radioactivity was measured in lymph and blood. Since, though, the pharmaca can be metabolized in the body to a certain extent, the radioactivity values found, in this case the 24 hr lymph absorption and the lymph / portal blood concentration, will not only be related to unmetabolized pharmacon, but will also include the metabolites. From 9 pharmaca the presence of metabolized and unmetabolized form in lymph- and blood samples was investigated.

- From the results, described in chapter 3, it appeared that the absorption of DDT via the lymph tracts was greatest after administration in a fatty dosage form. Therefore, in order that the absorption via the lymph tract should be as great as possible, the pharmaca were administered in 4 ml micellar solution or 0.3 ml triolein.

4.2. EXPERIMENTAL PROCEDURES

4.2.1. Animal experiments.

Two types of animal experiments have been carried out. The technical descriptions of these experiments can be found in chapter 2.3.2.

A. Animal experiments of the first type.

With the animal experiment of the first type radioactively labelled pharmaca were administered into the stomach. After that the radioactivity in the lymph samples, which were collected up to 24 hr after administration, was determined. Radioactivity was also determined in the faeces, collected during 24 hr, and in the intestinal contents, obtained after 24 hr and in the intestinal wall. It is assumed that the total radioactivity, obtained by summation of the activity in faeces, intestinal contents and intestinal wall, represents the non-absorbed

activity. Since the possibility exists that the activity, already absorbed in the body, is again excreted in a small measure with the bile into the intestine, we are concerned with an approximated value. By subtracting the activity obtained by the above summation from the administered activity, the "apparent absorbed activity" was obtained. The activities in the lymph samples were always calculated as percentages of the "apparent absorbed activity". These lymph samples were collected at 0-2, 2-4, 4-6, 6-8, 8-12 and 12-24 hr following administration. The activities measured in the samples are presented cumulatively. Moreover the activity of the lymph, collected during 24 hr is presented as a percentage of the administered dose. The activities in 24 hr urine and in the liver, perirenal fat and intracardial blood after 24 hr were also determined. The values found give an impression of the absorption of the pharmacon via the portal vein and the distribution in the investigated tissues and urine. Each pharmacon was tested in at least two rats.

As well as the experiments just described where the pharmaca were administered either in micellar solution, or in triolein, some experiments were carried out in which the absorption via the lymph tract was studied after administration of the pharmacon in an altered administration form.

B. Animal experiments of the second type.

After administration of the pharmaca, labelled with ^{14}C and ^{3}H, in triolein or micellar solution the samples of mesenteric lymph and portal blood were taken at ½, 1, 2, 3 and 4 hr following administration. The radioactivity per gram lymph or blood was calculated. With the help of these values the quotients of the activities in 1 g lymph / 1 g portal vein blood could be calculated. As has been described for the testing of a number of pharmaca, the lymph, that was collected from 3 hr 5 min until 3 hr 55 min after administration was used for the analytical determination of the percentage of unconverted pharmacon. This percentage was also determined in samples of portal blood which were collected approximately 4 hr following administration. This determination was carried out for the pharmaca vitamin D_3, DDT, DDE, dieldrin, quinestrol, mestranol, methylcholanthrene, cyclandelate and D.O.P. For all the investigated pharmaca, curves are given for the radio activities of the pharmacon in lymph and blood and for the lymph / portal blood concentration quotient after the time intervals mentioned. Each pharmacon was tested on 2 rats. Besides the above mentioned experiments where the pharmacon was administered into the stomach

in micellar solution or triolein, other experiments were carried out in which the dosage form was altered. In these experiments the percentage of unconverted pharmacon was also determined. In one experiment DDT was administered orally in 0.3 ml propylene glycol. In another group of experiments the pharmacon, dissolved in 0.1 ml propylene glycol, was injected into the tail vein of the rats. Two hr before this injection 0.3 ml triolein was administered, via the stomach cannula, to these rats. The last-mentioned experiments were carried out with the pharmaca vitamin D_3, DDT, dieldrin, quinestrol and mestranol.

4.2.2. Saponification of D.O.P. and cyclandelate in intestinal fluid.
The degree to which D.O.P. and cyclandelate are saponified in the intestine of the rat was studied by means of the following experiments in which saponification values were approximately determined. D.O.P. and cyclandelate were saponified in collected intestinal fluid of 2 rats. This intestinal fluid was obtained as follows: the rats were brought under halothane-N_2O narcosis. The abdomen was opened. At a distance of 5 cm from the stomach a ligature was applied around the small intestine. 4 ml of milk was injected into the stomach and the abdominal wall was closed again and the anesthesia terminated. After 1 hr the abdominal wall was again opened and a ligature was applied around the small intestine directly behind the stomach. The intestinal fluid from the piece of intestine, limited by the ligatures, was collected. From the 2 rats was collected 4 ml. This mixture was divided between 2 small containers and brought to 37 °C. 25 μg radioactive D.O.P., dissolved in 5 μl. of alcohol was added to one container; in the same manner cyclandelate was brought into the other, after which they were incubated for 1 hr. The percentages of unconverted product were determined in the same way as is discussed for a number of pharmaca in lymph and blood in the analytical section.

4.2.3. Investigated pharmaca.
In the animal experiments of the first type the pharmaca mentioned in table 1, were tested. These are the ^{14}C and ^{3}H labelled pharmaca, except for the mentioned cholesterol, phthalic anhydride and vitamin D^3. Moreover non-radioactive aroclor and hexachlorbiphenyl were investigated by means of these animal experiments.
In animal experiments of the second type the same pharmaca that are mentioned in table 1, were tested, except for the mentioned cholesterol and phthalic anhydride.

The ΔR_m value with respect to butter yellow was dertermined for the pharmaca mentioned under chapter 2 6 1. "Non-radioactive compounds", included triolein which is mentioned under the constituents of the vehicle

4 2 4 Analytical procedures

^{14}C and ^{3}H radioactivity were counted as described (chapter 2 7 2 1) For DDT, DDE dieldrin, quinestrol, mestranol, vitamin D_3, cyclandelate, D O P and methylcholanthrene the lymph, portal vein and aorta blood were investigated for percentage of unconverted pharmacon as follows Firstly the radioactivity was measured in two different parts (0 1-0 2 g) as is described in chapter 2 Subsequently an extraction according to Bligh and Dyer was employed (chapter 2 7 3) on the rest (1 g) The obtained chloroform residue contains the lipophilic pharmacon This chloroform residue was made up to 25 ml with chloroform in a measuring flask The incubate of intestinal fluid with D O P or cyclandelate was investigated in the same manner The blood- and intestine incubate samples were treated as follows 20 ml was reduced to a small residue by means of a stream of dry nitrogen and this residue was brought onto a reversed phase plate (chapter 2 7 4) as a line 5 cm long The lymph samples were treated in the same manner, except that a duplicate of 5 ml from the measuring flask was handled Pure radioactive pharmacon was pipetted onto 2 ml of chloroform from the flask and this sample, after reducing by evaporation, was applied on the plates as reference This procedure was followed for lymph and blood as well as for intestinal incubate All the plates were developed in a mixture of 60/40, v/v acetone/water The activity on the plates was measured as described The radioactivity of the peak was then multiplied by 25/20 for the blood samples and the intestinal fluid and by 25/5 for the lymph The activity obtained, divided by the calculated total activity of the sample, multiplied by 100 gave the percentage of unconverted pharmacon

4 2 5 Determination of the relative lipophilicity.

The determination of the degree of lipophilicity of the lipophilic pharmaca investigated by us as a partition coefficient between water and organic liquids is complicated by the extremely low solubility of these compounds in water and the high solubility in the organic liquids (Boyce and Milborrow, 1965, Van der Kleijn, 1969) Better results are obtained with the help of reversed phase chromatography With this the so-called ΔR_m value is obtained (Green and Marcinkiewicz, 1963) The

following theoretical relationship between partition-coefficient (α) and R_f value from liquid-liquid partition chromatography is deduced by Martin (1949)

$$\alpha = K\,(1/R_f - 1)$$

where K = constant for the system.

Bate-Smith and Westall (1950) introduced the term R_m, where

$$R_m = \log\,(1/R_f - 1)$$

So the relative lipophilicity, ΔR_m, of a pharmacon is finally expressed by

$$\Delta R_m = \log\,(1/R_{f,X} - 1) \,/\, (1/R_{f,R} - 1)$$

where $R_{f,X}$ is the R_f value of the pharmacon and

$R_{f,R}$ is the R_f value of the reference compound.

We dertermined the ΔR_m values according to the above method as follows: The pharmaca were placed on plates that were respectively developed with the developing liquids acetone/water v/v 30/70, 35/65, 40/60 etc. up to 85/15. This was done for each pharmacon for each developing liquid in a multiple of six in so far as the R_f value was > 0 and < 0.8. The calculated average R_m values were plotted against the composition of the developing liquids; the points found generated straight lines. Using the developing liquid acetone/water 60/40 an R_m value was obtained which occurred on the straight line of the pharmacon concerned in most cases. By means of this developing liquid the ΔR_m value of the pharmaca with respect to butter yellow was determined. Butter yellow was placed on each plate as a reference. The calculation of the ΔR_m value was carried out according to the deduced formula:

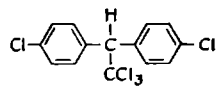
$$\Delta R_m = \log x'/x.\, h/h'$$

where x and h represent the distance travelled by respectively pharmacon and butter yellow, while x' and h' respectively represent the

distance of pharmacon and butter yellow from the front of the mobile liquid phase The averages and standard deviations obtained from 6 values are described below with the systematic treatment of the pharmaca. Exceptions were cortisone and dicumarol, which showed a much smaller lipophilicity than the other pharmaca. By extrapolation their values were determined at $< -0\,39$. Hexadecane, vitamins A, E, K_1, cholesterol, triolein and β sitosterole were also exceptions, showing a much greater lipophilicity Their values were determined at > 1.23 by extrapolation. The pharmaca were brought on the plate in quantities of 3 μg or less. The stains were detected in 3 ways as described in chapter 2.7.4 1.

4 3. RESULTS

4.3.1. DDT.



The extensive results from the animal experiment of the first type can be found in chapter 3 The following experiment was also carried out employing the animal experiment of the first type, in order to examine the influence of the administration site of the pharmacon on the extent of lymphatic resorption (see table 4): 0.3 ml triolein was administered into the stomach of two rats 2 hours before administration of ^{14}C-DDT. ^{14}C-DDT was dissolved in 0 1 ml propylene glycol and injected into the tail vein In the usual way, lymph samples, intestine and faeces were collected and the ^{14}C activity in these was determined. The extent of absorption via the lymph appeared to be small.

Table 4 ^{14}C radioactivity, measured in lymph, faeces and gut after i v. injection of ^{14}C-DDT* in the tail of the rat

rat	administered		percentage of the administered amount collected in thoracic duct lymph after hr						faeces and gut after 24 hr
	amount	act	0-2	2-4	4-8	8-12	12-24	0-24	
a	1 mg	2 5 μCi	0 02	< 0 01	0 09	0 09	0 20	0 40	0 5
b	1 mg	2 5 μCi	0 02	0 03	0 10	0 06	0 09	0 30	0 5

* ^{14}C-DDT was administered in 0 1 ml propylene glycol, 2 hr before the injection of ^{14}C-DDT 0 3 ml triolein was administered in the stomach of the rats

The results of the animal experiment of the second type are shown in fig. 8. With these experiments ^{14}C-DDT was not only administered orally in micellar solution to two rats but also in propylene glycol. In addition, ^{14}C-DDT was administered i.v. dissolved in propylene glycol to 1 rat via the tail. In this last mentioned case, 0.3 ml of triolein was brought into the stomach 2 hours before the administration.
In the experiments described in table 4 and fig. 8 total ^{14}C radioactivity was measured and no differentiation has been made between the parent compound and the metabolites. In order to obtain an impression of the percentage of DDT which was metabolized, samples of lymph and portal blood were taken in the period of maximal absorption (at 3-4 hr after oral administration and at ½ - 1 hr after i.v. administration). The samples were analyzed for the presence of unmetabolized DDT. The values are given in table 5.

Table 5. The presence of unmetabolized DDT in mesenteric lymph and portal blood at 3-4 hr after oral administration (at ½-1 hr in case of i.v. administration). The values are presented as percentages of total ^{14}C radioactivity.

rat	dosage form	route of administration	unmetabolized pharmacon (%)	
			lymph	portal blood
1	micel. sol.	p.o.	97	72
2	micel. sol.	p.o.	97	72
3	prop. glyc.	i.v.	80	83

Reversed phase chomatography revealed a ΔR_m value of 0.38 (s.d. 0.01). Non-radioactive DDT was detected with U.V. absorption.

Discussion:
In chapter 3 an investigation concerning the degree of absorption via the lymph, dependent on the oral dosage form, was carried out. It appeared that DDT was absorbed to a considerably greater extent via the lymph tract following administration in a fatty dosage from (approx. 50 per cent of the administered quantity), than following administration in the non-fatty dosage form propylene glycol (approx. 13 per cent of the administered quanatity). The absorption via the portal vein was calculated from the results, but was not directly measured. Following

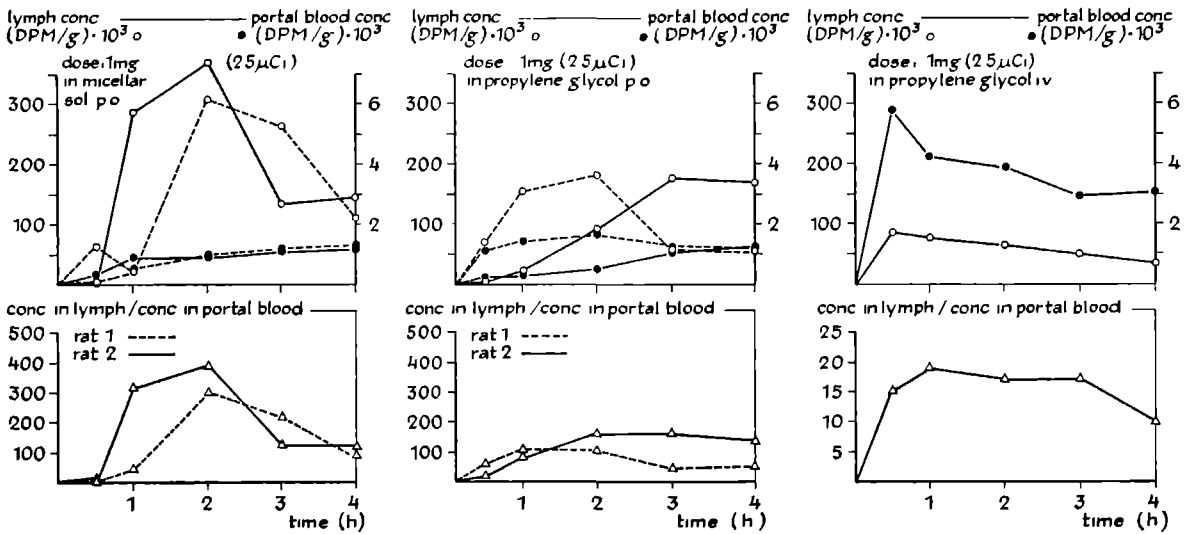


Fig 8

Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of DDT and the corresponding quotients of these concentrations The concentrations are given as disintegrations per minute (DPM) per gram In the case of i v administration 0 3 ml triolein was given 2 hr before into the stomach

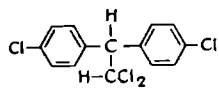
the animal experiment of the second type described here, results were found (see fig. 8) which can be seen to confirm the results described in the 3rd chapter. The portal vein blood indeed appears to contain ^{14}C-DDT and the concentration of DDT in lymph in respect of portal vein blood appears to vary as a function of the dosage form. In the introduction to this chapter the fact that the flow ratio of portal vein blood and thoracic duct lymph, post absorption, is approximately 500 in the rat (Landis, 1962) has been discussed. If there is a considerable transport of DDT via the lymph tract, then the quotient of the concentrations of DDT in lymph and portal vein blood must also be considerable. This indeed appears to be the case. Following oral administration in micellar solution this quotient appears to show peak values of approx. 300 and 400 respectively, whereas these peak values are approx. 100 and 170 respectively, following oral administration of DDT in propylene glycol. These results are analogous to the differences in absorption via the lymph during 24 hours following administration of DDT in fatty and non-fatty dosage form. Following oral administration in fat, DDT appears after absorption from the intestine, in the mucosa cells and can be absorbed in the chylomicrons and other lipoproteins, which have been formed there. This mechanism may be the explanation for the relatively large DDT transport. In order to examine whether oral administration is necessary for a relatively large transport of DDT via the lymph ^{14}C-DDT was dissolved in propylene glycol and injected into the tail vein of the rat. Subsequently DDT was detected in lymph and portal vein blood samples. By administering triolein into the stomach, 2 hours previously in this experiment, we made certain that, at the moment of injection, the lymph tract was transporting a considerable quantity of chylomicrons. In table 4 it can be seen that in this case the lymphatic transport of the DDT administered in this way was very small over 24 hours (< 0.5 per cent).

The conclusion may be drawn that the vehicle as well as the site of administration are important for the extent of lymphatic transport. One could conclude that little exchange of DDT beween lymph and blood occurs. If this were occurring to any great extent, then after oral administration one would not be able to find such high values for the lymphatic transport of DDT over 24 hours (see chapter 3). These results are also confirmed in fig. 8. The concentration of ^{14}C-DDT in the portal vein blood after i.v. administration appears to be large in respect to the other curves obtained after oral administration. The lymph concentration is also low after i.v. administration and the quotient in lymph/

portal vein blood even appears to be < 20 in the peak values. The results mentioned were obtained by measurement of the ^{14}C activity following administration of ^{14}C-DDT. Table 5 shows that to a small extent metabolites are present in lymph and portal vein blood. It is striking that following oral administration the percentage metabolite in blood is greater than in lymph.

The metabolites DDD and DDE from DDT show a considerable lipophilicity, as does DDT, but DDE especially shows a completely different configurational structure. Whilst DDT and DDD show a tetrahedron structure, DDE has a y-shape. Because of the possible connection of the structure of the pharmaca with the extent of lymphatic absorption, both pharmaca were investigated.

4.3.2. DDD.



The results from the animal experiment of the first type (chapter 2) are shown in fig. 9 and table 6.

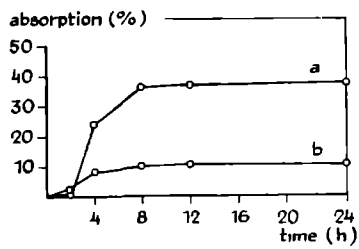


Fig 9
Cumulative absorption of DDD in thoracic duct lymph The lymphatic absorption is presented as a percentage of the apparent absorption. Apparent absorption means the amount of the administered radioactive pharmacon minus the amount of radioactivity present in faeces and gut after 24 hr; a, b (etc.) refer to different rats.

Table 6. Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}C-DDD.

rat	dosage form	administered amount (mg)	administered act (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat.
a	triolein	1	¾	16 9	1 3	31.1	1 3	0 02	2.0
b	mic sol	1	¾	14 3	0 2	8 9	1 8	0 04	1 2

The results from the animal experiment of the second type (chapter 2) are shown in fig. 10.

The ΔR_m value in respect of butter yellow was found to be 0.26 (s.d. 0.03). Non-radioactive DDD was detected using UV absorption.

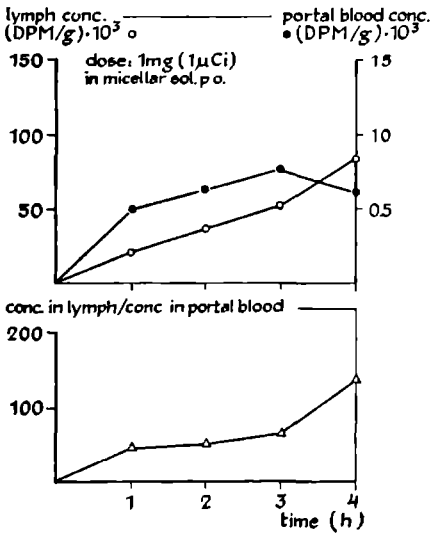
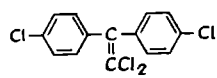


Fig. 10
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of DDD and the corresponding quotients of these concentrations.
For further details see legend of fig. 8.

4.3.3. DDE.



The results from the animal experiment of the first type (chapter 2) are shown in fig. 11 and table 7.

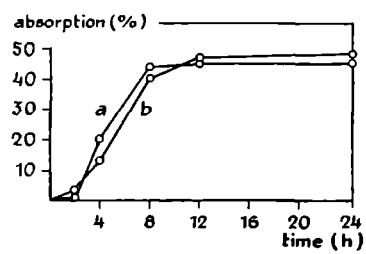


Fig. 11
Cumulative absorption of DDE in thoracic duct lymph.
For further details see legend of fig. 9.

Table 7. Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}C-DDE.

rat	dosage form	administered amount (mg)	administered act. (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat.
a	triolein	1	1	8.5	0 12	41.8	1.3	0.005	2.2
b	mic. sol.	1	1	16 0	0 05	40 8	0.7	0 001	1.1

The results from the animal experiment of the second type (chapter 2) are shown in fig. 12.
The percentage of unmetabolized DDE at 3-4 hours following oral administration is given in table 8.
The ΔR_m value in respect of butter yellow was found to be 0.44 (s.d. 0.01). DDE was detected using UV absorption.

Discussion:
DDD and DDE appear to be absorbed, similarly to DDT, via the lymph tract, to a considerable extent. DDE, which has a different configurational structure – namely a y-shape, whereas DDT has a tetrahedron structure- but of which the ΔR_m value is approximately equal to that of

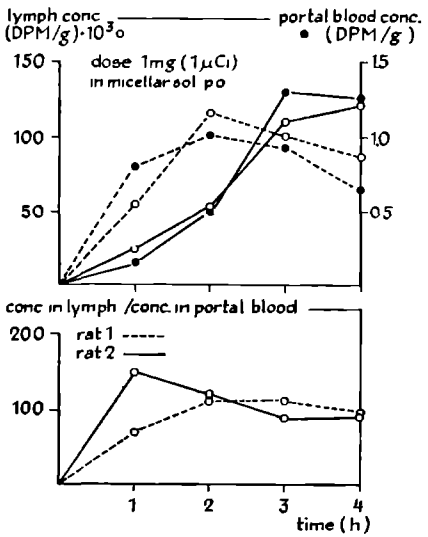


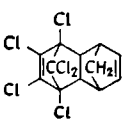
Fig. 12
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of DDE and the quotients of these concentrations.
For further details see legend of fig 8.

Table 8. The presence of unmetabolized DDE in mesenteric lymph and portal blood at 3-4 hr after oral administration (at ½-1 hr in case of i.v. administration). The values are presented as percentages of total ^{14}C radioactivity.

rat	dosage form	route of administration	unmetabolized pharmacon (%)	
			lymph	portal blood
1	triolein	p o	97	88
2	micel sol	p o	93	83

DDT (resp. 0.44 and 0.38), appears in these experiments to be absorbed via the lymph tract to approx. the same extent (approx. 45-50 per cent of the quantity, absorbed from the intestine). Similar to DDT, the fatty tissue appears to bind a considerable amount of DDD and DDE (see table 6 and 7).

Aldrin and its metabolite, the epoxide dieldrin (Wong and Terriere, 1965) are also strongly lipophilic (Heath and Vandekar, 1964). Therefore these pharmaca have also been investigated.



4.3.4. ALDRIN.

The results from the animal experiment of the first type are shown in fig. 13 and table 9. Triolein as well as propylene glycol was used as oral administration form.

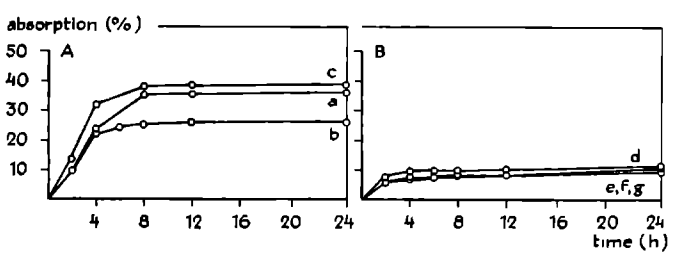


Fig. 13
Cumulative absorption of aldrin in thoracic duct lymph A in triolein, B in prop glyc. For further details see legend of fig 9

Table 9 Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}C-aldrin

rat	dosage form	administered amount (mg)	administered act (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat
a	triolein	1	1	20 5	0 7	28 5	4 7	0 012	3 5
b	triolein	1	1	8 9	0 15	23 5	1 7	0 001	1 6
c	triolein	1	1	34 3	—	25 2	3 3	0 004	2 7
d	prop glyc	1	1	23 6	1 3	8 0	3 2	0 02	3 7
e	prop glyc	1	1	33 2	1 0	5 9	3 3	—	4 3
f	prop glyc	1	1	27 6	1 3	6 6	5 0	0 03	5 3
g	prop glyc	1	1	27 3	0 8	7 0	4 3	0 02	4 0

The results from the animal experiment of the second type are shown in fig 14

ΔR_m value in respect of butter yellow of 0 60 (s d. 0 15) was obtained Non-radioactive aldrin was detected by spraying the chromatography plates with $KMnO_4$ (chapter 2)

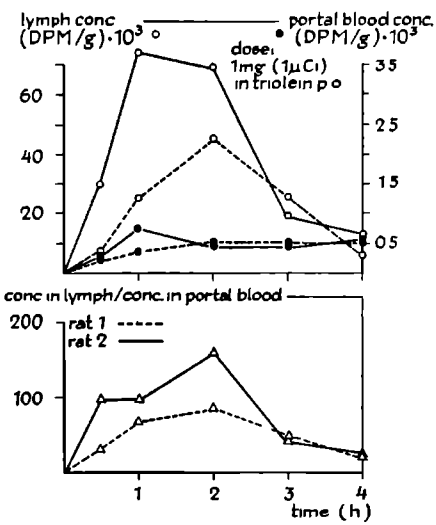
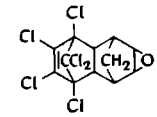


Fig 14
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of aldrin and the corresponding quotients of these concentrations
For further details see legend of fig 8



4.3.5. DIELDRIN.

The results from the animal experiment of the first type are shown in fig. 15 and table 10.

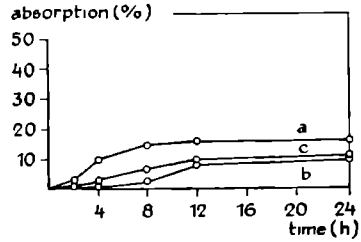


Fig. 15
Cumulative absorption of dieldrin in thoracic duct lymph
For further details see legend of fig 9

Table 10. Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}C-dieldrin.

rat	dosage form	administered amount (mg)	administered act (μCi)	percentage of the administered amount of radioactivity: for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat
a	triolein	1	1	14 8	0 3	13 7	2 7	0 015	4 7
b	mic sol	1	¾	3 8	0 8	8 9	2 0	0 003	2 7
c	mic sol.	1	1	12 8	0 3	9 4	2 2	0 013	3 7

The results from the animal experiment of the second type are shown in fig. 16.
The percentage of unmetabolized dieldrin in lymph and portal vein blood, 3-4 hr following oral administration and ½-1 hr following administration into the tail vein is shown in table 11.
0.24 (s.d. 0.01) was obtained as ΔR_m value in respect of butter yellow.
^{14}C-dieldrin was detected using a radioactivity scanner (see chapter 2.7.2.2).

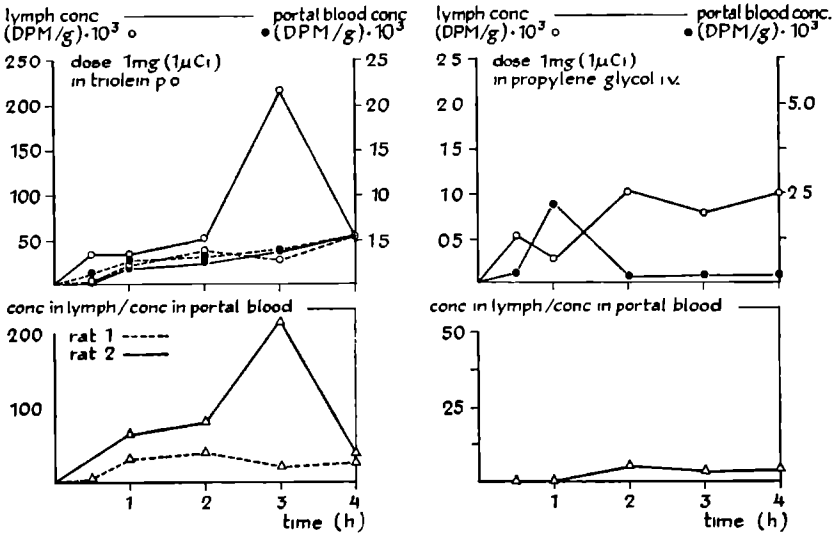


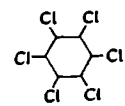
Fig 16
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of dieldrin and the corresponding quotients of these concentrations.
For further details see legend of fig 8

Discussion:
In view of the experiments, which will be described in chapter 6, aldrin was administered in triolein as well as in propylene glycol. Like DDT (chapter 3) aldrin appears to be absorbed via the lymph tract more when administered in a fatty dosage form than when in a non-fatty

Table 11. The presence of unmetabolized dieldrin in mesenteric lymph and portal blood at 3-4 hr after oral administration (at ½-1 hr in case of i.v. administration). The values are presented as percentages of total ^{14}C radioactivity.

rat	dosage form	route of administration	unmetabolized pharmacon (%)	
			lymph	portal blood
1	triolein	p o.	98	85
2	triolein	p o.	92	85
3	prop. glyc.	i.v	96	51

dosage form (respectively about 33 and 10 per cent of the apparent absorption). Although the ΔR_m value of aldrin is 0.60 (s.d. 0.15) and that of DDT is 0.38 (s.d. 0.01) it appears in these experiments that aldrin is less absorbed via the lymph tract following oral administration in fat (approx. 33 per cent of the apparent absorption). The lymphatic absorption is considerable for both, though.
The ΔR_m value of dieldrin - 0.24 (s.d. 0.01) - is smaller than that of aldrin - 0.60 (s.d. 0.15) -. The apparent absorption of dieldrin after administration in fatty dosage (about 12 per cent) is also smaller than that of aldrin (about 33 per cent). Like DDT, dieldrin, following injection into the tail vein whilst the rat is absorbing triolein, appears to show a low concentration quotient between lymph/portal vein blood, compared to this quotient following oral fatty administration (see fig. 16). Aldrin and dieldrin both appear to be stored to a great extent in the fatty tissue (see table 9 and 10). The percentage of unconverted dieldrin in lymph appears to be greater than in blood, as it also is for DDT (see table 11).



4.3.6. LINDANE.

The results from the animal experiment of the first type are shown in fig. 17 and table 12.

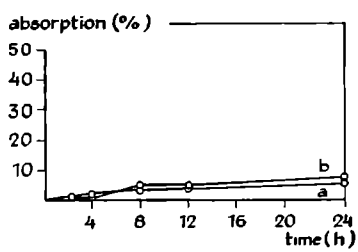


Fig. 17
Cumulative absorption of lindane in thoracic duct lymph.
For further details see legend of fig. 9.

The results from the animal experiment of the second type are shown in fig. 18.
0.06 (s.d. 0.03) was obtained as ΔR_m value in respect of butter yellow. Detection was carried out using scanning of the radioactive product.

Table 12. Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}C-lindane.

rat	dosage form	administered amount (mg)	administered act (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat
a	triolein	2	1	15 0	15 8	4 3	2 3	—	—
b	mic sol.	2	1	18.3	21.6	5.9	2 2	0 02	0.7

Discussion:

The ΔR_m value of lindane in respect of butter yellow, 0.06 (s.d. 0.03), is smaller than that of the previously considered pharmaca. The apparent absorption via the lymph tract during 24 hours (approx. 6 per cent) and the quotient of the concentration in lymph/portal vein blood (approx. 6 at most) also appear to be smaller. The pharmacon and its metabolites are stored less in the fatty tissue and excreted more into the urine than is the case for the previously considered pharmaca.

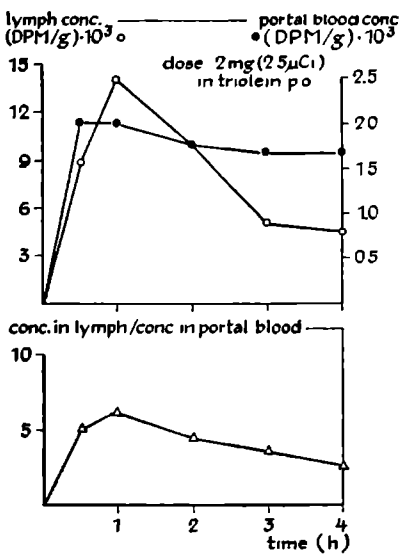
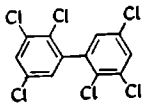


Fig. 18
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of lindane and the corresponding quotients of these concentrations
For further details see legend of fig 8

4.3.7. HEXACHLORBIPHENYL AND AROCLOR



Aroclor is an industrial product. It is a mixture of polychlorbiphenyl-isomers (PCBs).
By way of exception these pharmaca were not radioactive. Only the animal experiment of the first type was carried out and with that only the thoracic duct lymph was analysed. This was done by strongly shaking 1 ml lymph for 10 min with 25 ml hexane. After separation of the layers 5 microliters of the hexane extract was injected into the gas chromatograph (with E.C.-detector). The following column was used: glass, 6′ x ¼″, filled with 3 per cent OV-1 on Gas Chrom. Q, 80-100 Mesh. Temperature: 190 °C, carrier-gas: N_2 approx. 70 ml/min.
Data from the animal experiment of the first type; 1 mg of hexachlorbiphenyl as well as 1 mg of aroclor were separately administered to each of 2 rats. The thoracic duct lymph was collected and the percentage of both products was determined. 39.9 per cent of the administered quantity of hexachlorbiphenyl and 40.0 per cent of the aroclor were recovered in the 24 hours lymph.
0.53 (s.d. 0.01) was obtained as ΔR_m value of hexachlorbiphenyl in respect of butter yellow. It was detected by means of UV fluorescence.

Discussion:
Aroclor is a technical product employed in various processes among others in dying, and occurs eventually as an environmental pollutant (Koeman et al, 1969). It appears to have many properties in common with DDT, which is also a chlorinated hydrocarbon, among others may be mentioned that it is stored in the body fats for a long time (Pichirallo, 1971) .If the absorption of hexachlorbiphenyl is similar to DDT which is nearly 100 per cent, then the "apparent absorption" via the lymph tract can also be taken to be 39.9 per cent. The relative heights of the 12 peaks obtained from aroclor components in the chromatogram of the lymph sample did not differ from those of the aroclor standard. These aroclor components are PCB-isomers with respectively 6, 7 and 8 chlorine atoms per molecule. This result again suggests that the lipophilicity of the isomers plays a large role in lymphatic transport.

The results from the animal experiment of the first type are shown in fig. 19 and table 13. 0.3 ml Triolein as well as 0.3 ml liquid paraffin was used as dosage form.

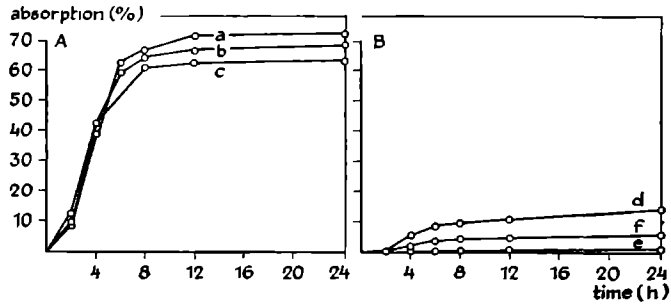


Fig. 19
Cumulative absorption of hexadecane in thoracic duct lymph A· in triolein, B· in liquid paraffin
For further details see legend of fig. 9

Table 13. Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}C-hexadecane.

rat	dosage form	administered amount (mg)	administered act (μCi)	percentage of the administered amount of radioactivity for 24 hr in: faeces and gut	urine	lymph	after 24 hr in: total liver	1 g cardiac blood	1 g perirenal fat.
a	triolein	20	1 5	31 0	0 4	50 0	0 4	0 004	0 015
b	triolein	20	1 5	28 2	0 5	49 1	0 3	0 005	0 014
c	triolein	20	1 5	30 4	0 45	44 1	0 3	0 004	0 014
d	0 3 ml liquid paraffin	20	1	65 4	0 14	5 0	0 13	< 0 001	0 032
e	0 3 ml liquid paraffin	20	1	41 5	0 05	0 5	0 02	< 0 001	< 0 001
f	0 3 ml liquid paraffin	20	1	72 1	0 1	1.4	0 05	< 0 001	0 01

The results from the animal experiment of the second type are shown in fig. 20.
The ΔR_m value appeared to be so large that this could only be found to be > 1.23 by means of extrapolation. Detection was achieved by scanning the radioactive compound.

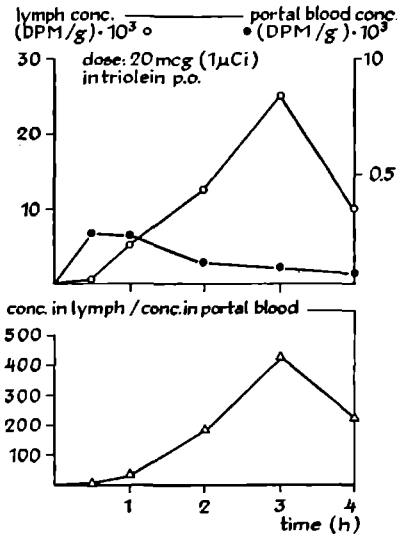
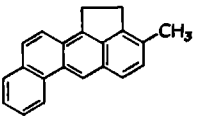


Fig. 20
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of hexadecane and the corresponding quotients of these concentrations.
For further details see legend of fig. 8.

Discussion:
Hexadecane is a component of liquid paraffin. This contains the series of saturated aliphatic hydrocarbon chains from C_{14} up to C_{26}. Until now it was assumed that liquid paraffin is not absorbed from the intestine. From the experiments described here, it appears that administration in triolein to the rat results in a considerable absorption, whereas administration in liquid paraffin results in a variable but small absorption. We must bear in mind, though, that liquid paraffin is used as a laxative, naturally in cases where emptying of the intestine is incomplete. In such cases the fat, still present in the intestine, could promote the absorption of liquid paraffin. By analogy with the above mentioned compounds one would expect a considerable accumulation of hexadecane in fat. This, though, appears not to occur. It seems probable, that this material is metabolized.



4.3.9. 3-METHYLCHOLANTHRENE

The results from the animal experiment of the first type are presented in fig. 21 and table 14.

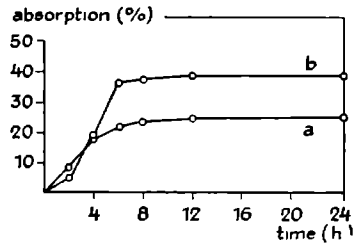


Fig. 21
Cumulative absorption of 3-methylcholanthrene in thoracic duct lymph
For further details see legend of fig 9

Table 14. Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}C-metylcholanthrene.

rat	dosage form	administered amount (mg)	administered act (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat
a	triolein	1	1	40 5	6 5	14 6	0.7	0 008	0 027
b	triolein	1	1 5	51 0	—	18 8	1.0	0 022	0 023

The results from the animal experiment of the second type are shown in fig. 22.
The percentage of unmetabolized methylcholanthrene at 3-4 hr following oral administration is given in table 15.
0.25 (s.d. 0.03) was obtained as ΔR_m value in respect of butter yellow.
Detection was carried out by spraying with $KMnO_4$.

Discussion:
Methylcholanthrene is an aromatic hydrocarbon with carcinogenic activity. Its ΔR_m value in respect of butter yellow (0.25) is identical to that of dieldrin (0.24) and its lymphatic transport is considerable,

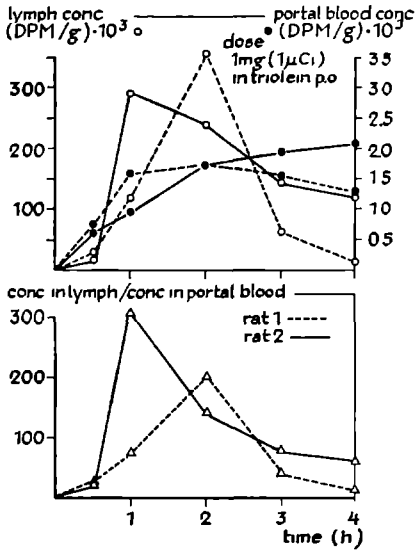


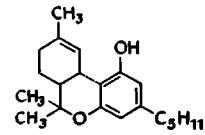
Fig 22
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of 3-methylcholanthrene and the corresponding quotients of these concentrations
For further details see legend of fig. 8.

(24-38 per cent of the apparent absorption). In thoracic duct lymph, concentrations up to 200-300 times greater than the concentration in portal vein blood are reached (fig. 22). The question arises whether, due to high concentration of the transported methylcholanthrene, carcinomas develop to a greater extent in the walls of the lymph vessels and lymph nodes in the intestinal area and in the thoracic duct. Against all expectations this compound, like hexadecane, is slightly absorbed into body fat. Here the percentage of metabolite is also larger in blood than in lymph (table 15).

Table 15. The presence of unmetabolized 3-methylcholanthrene in mesenteric lymph and portal blood at 3-4 hr after oral administration (at ½-1 hr in case of i.v. administration). The values are presented as percentages of total ^{14}C radioactivity.

rat	dosage form	route of administration	unmetabolized pharmacon (%)	
			lymph	portal blood
1	triolein	p.o.	83	70
2	triolein	p.o.	85	49

4.3.10. Δ 1-TETRAHYDROCANNABINOL



The results of the animal experiment of the first type are shown in fig. 23 and table 16.

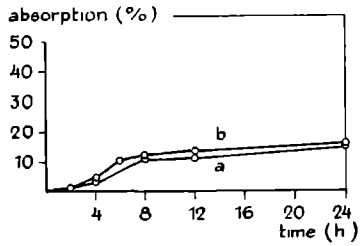


Fig. 23
Cumulative absorption of Δ 1-tetrahydrocannabinol in thoracic duct lymph.
For further details see legend of fig. 9.

Table 16. Radioactivity measured in several biological fluids and tissues after oral administration of ^{3}H-Δ 1-tetrahydrocannabinol.

rat	dosage form	administered amount (mg)	act. (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	urine	lymph	after 24 hr in total liver	1 g cardiac blood	1 g perirenal fat.
a	triolein	0 020	10	32.2	11.9	9.8	1.5	0.11	0.13
b	mic. sol.	0.020	10	36 8	13.0	9.8	0.9	0.10	0.07

The results of the animal experiment of the second type are shown in fig. 24.
0.43 (s.d. 0.03) was obtained as Δ R_m value in respect of butter yellow. Detection was achieved by scanning the radioactive compound.

Discussion:
Δ 1-THC is an active primary constituent of marihuana. The Δ R_m value of Δ 1-THC (0.43) is greater than that of DDT (0.38). The transport via the lymph tract is considerable (approx. 15 per cent of the apparent absorption) but not as large as from DDT (approx. 50 per cent). The storage in perirenal fat is small. A relatively large excretion via the urine (see table 16) is explicable from the fact that this lipophilic pharmacon is to a considerable extent converted in the body to more hydrophilic products (Vree et al, 1972).

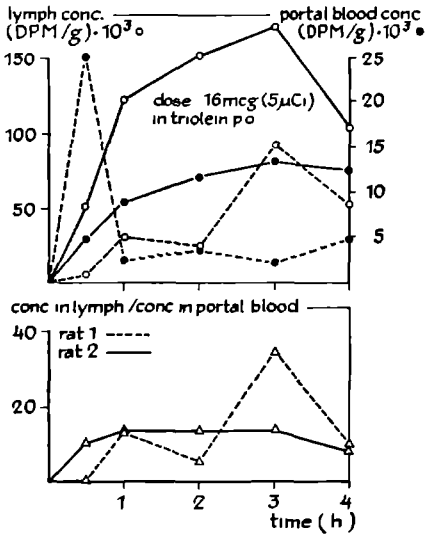
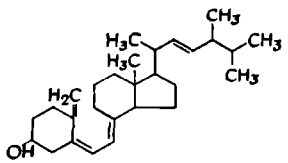


Fig 24
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of Δ 1-tetrahydrocannabinol and the corresponding quotients of these concentrations
For further details see legend of fig 8.



4.3.11. VITAMIN D_3

The animal experiment of the first type has not been carried out by us. In the literature one can find results of analogous experiments. Schachter et al (1964) found after oral administration in a fatty vehicle absorption via the intestinal lymph tract of 75-100 per cent of the apparent absorption.

Results of the animal experiment of the second type are shown in fig. 25.

The percentage of unconverted pharmacon 3-4 hr following oral administration and ½-1 hr following administration into the tail vein is shown in table 17.

1.23 (s.d. 0.12) was obtained as ΔR_m value in respect of butter yellow. Detection was carried out by means of spraying with $KMnO_4$.

Discussion:

The large lymphatic transport and the large concentration quotient of vitamin D_3 in lymph/portal vein blood agree with a high ΔR_m value of vitamin D_3 in respect of butter yellow. Now we see again that the con-

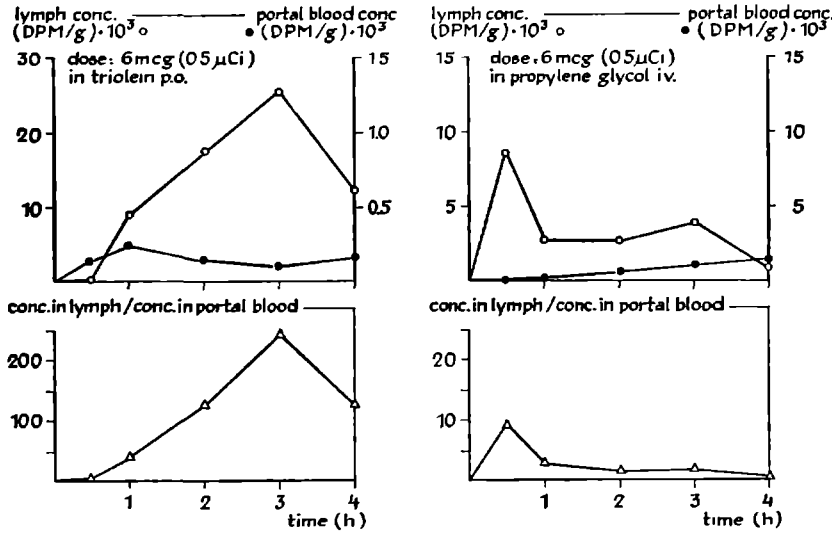


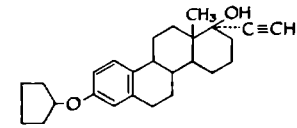
Fig. 25
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of vitamin D_3 and the corresponding quotients of these concentrations.
For further details see legend of fig 8.

centration quotient of vitamin D_3 in lymph/portal vein blood following intravenous administration in propylene glycol, when 0.3 ml triolein has been orally administered 2 hr previously, is small (approx. 9) compared to this quotient (peak value approx. 244) obtained following oral administration. Also the quantity of metabolite in portal vein blood is greater here than in lymph.

Table 17. The presence of unmetabolized vitamin D_3 in mesenteric lymph and portal blood at 3-4 hr after oral administration (at ½-1 hr in case of i.v. administration). The values are presented as percentages of total ^{14}C radioactivity.

rat	dosage form	route of administration	unmetabolized pharmacon (%)	
			lymph	portal blood
1	triolein	p.o.	90	27
2	prop. glyc.	i v.	86	57

Vitamin D_3 has a structural affinity with cholesterol. Because of this structural affinity with sterols and steroids, the steroids quinestrol, mestranol, progesterone, nandrolone and lynestrenol are now considered.



4.3.12. QUINESTROL

The results of the animal experiment of the first type are shown in fig. 26 and table 18.

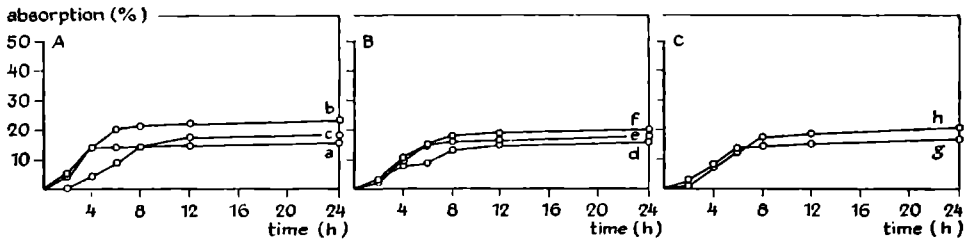


Fig. 26
Cumulative absorption of quinestrol in thoracic duct lymph. A: in triolein; B: in mic. sol.; C: in mono- and triolein (25/75).
For further details see legend of fig. 9.

The results of the animal experiment of the second type are shown in fig. 27.
The percentage of unconverted pharmacon 3-4 hr following oral administration and ½-1 hr following administration into the tail vein is shown in table 19.
0.24 (s.d. 0.03) was obtained as ΔR_m value in respect of butter yellow. Detection was carried out using $KMnO_4$ spraying.

Discussion:
The ΔR_m value in respect of butter yellow for quinestrol (0.24) is equal to that of dieldrin (0.24). The extent of lymphatic transport of the pharmaca is nearly identical. Other authors have investigated the extent of absorption of quinestrol via the thoracic duct (Giannina et al, 1967). They found a higher lymphatic transport of quinestrol when administered in sesame-oil, containing 25 w/v per cent monoolein, than after administration in sesame-oil alone. We can confirm their results for the triolein administration form. In our investigation it is approx. 10

Table 18. Radioactivity measured in several biological fluids and tissues after oral administration of ^{3}H-quinestrol.

rat	dosage form	administered amount (mg)	administered act (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat
a	triolein	0 020	5	37 0	7 5	9 8	2 6	0 02	0 3
b	triolein	0 020	5	50 9	6 5	11 4	3 5	0 03	0 3
c	triolein	0 020	5	45 1	2 0	10 1	1 8	0 03	0 3
d	mic sol	0 020	5	53 5	5 9	7 0	1 4	0 01	0 25
e	mic sol	0 020	10	48 2	5 6	8 8	1 9	0 02	0 3
f	mic sol	0 020	10	48 6	2 5	10 1	1 8	0 04	0 5
g	mono- and triolein, 25/75	0 020	5	53 1	8 5	7 9	3 2	0 03	0 3
h	mono- and triolein, 25/75	0 020	5	60 7	2 8	8 2	3 0	0 02	0 3

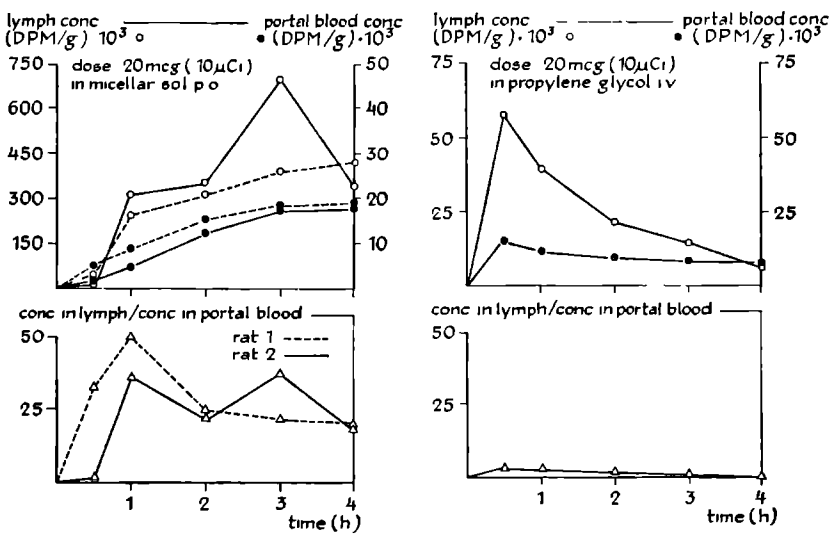


Fig 27

Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of quinestrol and the corresponding quotients of these concentrations

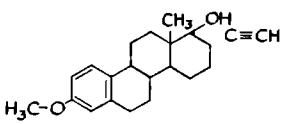
For further details see legend of fig 8

Table 19 The presence of unmetabolized quinestrol in mesenteric lymph and portal blood 3-4 hr after oral administration (at ½-1 hr in case of i v administration) The values are presented as percentages of total ^{14}C radioactivity

rat	dosage form	route of administration	unmetabolized pharmacon (%)	
			lymph	portal blood
1	micel sol	p o	93	43
2	micel sol	p o	83	43
3	prop glyc	i v	81	45

per cent of the administered quantity in lymph (table 18), in their results, after administration in sesame-oil, the value was 7 5 per cent of the administered quantity They found 15 7 per cent of the administered quantity in the thoracic duct lymph, following administration in sesame-oil, containing 25 w/v per cent monoolein, we do not find this increase following administration in triolein/monoolein 75/25 w/w We also did not obtain a lymphatic transport increase by administration in micellar solution Here again the percentage metabolite is greater in blood than in lymph (table 19), also the concentration quotient of quinestrol in lymph/portal vein blood is smaller following i v administration of quinestrol (peak value approx 4) than following oral administration (peak value 40-50)

4 3 13 MESTRANOL



The results of the animal experiment of the first type are shown in fig 28 and table 20

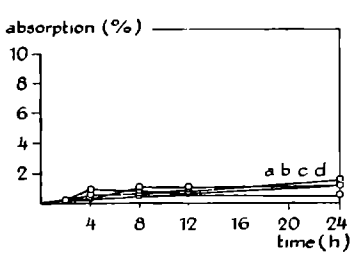


Fig 28
Cumulative absorption of mestranol in thoracic duct lymph
For further details see legend of fig 9

Table 20. Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}C-mestranol.

rat	dosage form	administered amount (mg)	administered act (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat
a	mic sol	0 030	1	56 7	—	0 67	0 6	—	0 012
b	mic sol	0 030	1	15 7	3 6	0 46	0 7	0 008	0 017
c	mic sol	0 030	2	67 0	—	0 35	—	—	—
d	mic sol	0 030	1	18 5	7 0	0 95	0 1	0 001	0 006

The results of the animal experiment of the second type are shown in fig 29.

The percentage of unconverted pharmacon, 3-4 hr following oral administration and ½-1 hr following administration into the tail vein is shown in table 21.

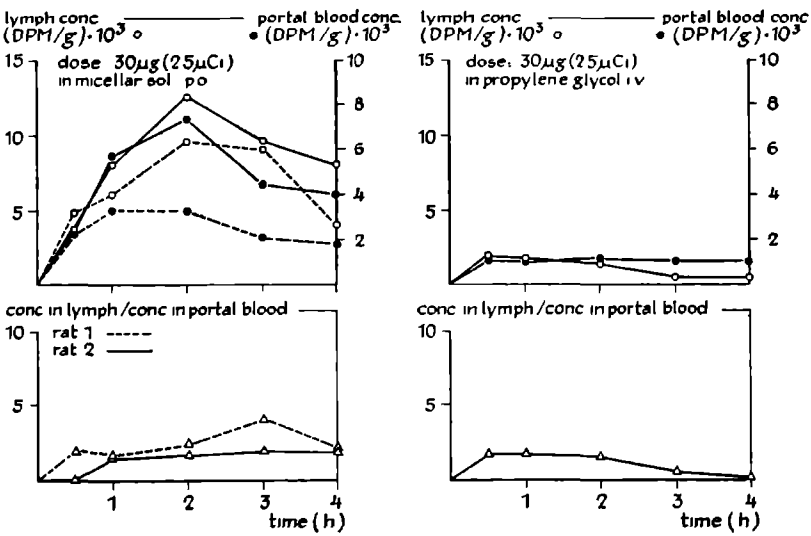


Fig 29

Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of mestranol and the corresponding quotients of these concentrations

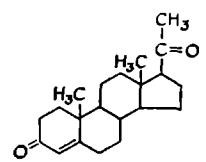
For further details see legend of fig 8

Table 21 The presence of unmetabolized mestranol in mesenteric lymph and portal blood at 3-4 hr after oral administration (at ½-1 hr in case of i v. administration). The values are presented as percentages of total ^{14}C radioactivity.

rat	dosage form	route of administration	unmetabolized pharmacon (%)	
			lymph	portal blood
1	micel sol	p o	72	26
2	micel sol.	p o	70	14
3	prop glyc	i v	64	37

0.08 (s.d. 0 06) was found as ΔR_m value in respect of butter yellow. Detection was carried out using $KMnO_4$ spraying.

Discussion:
Quinestrol is the cyclopentylether of ethynyl estradiol. Mestranol is the methylether of ethynyl estradiol. So the compounds only differ in the cyclopentyl and in the methyl component The ΔR_m value descreases considerably due to this, though. We also find a small apparent absorption via the thoracic duct lymph and a small concentration quotient in lymph/portal vein blood. The percentage of metabolite is again greater in blood than in lymph (table 21).



4.3.14. PROGESTERONE

The results of the animal experiment of the first type are shown in fig. 30 and table 22.

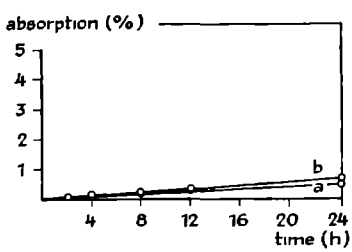


Fig 30
Cumulative absorption of progesterone in thoracic duct lymph
For further details see legend of fig 9

Table 22. Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}C-progesterone.

rat	dosage form	administered amount (mg)	administered act (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat.
a	mic sol	4	1	36 1	3 9	0 27	0 11	< 0 001	< 0 001
b	triolein	4	1	47 0	6 9	0 37	0 4	< 0 001	0 006

The results of the animal experiment of the second type are shown in fig. 31.
The ΔR_m value in respect of butter yellow was found to be -0.18 (s.d. 0.05). Detection was carried out using $KMnO_4$ spraying.

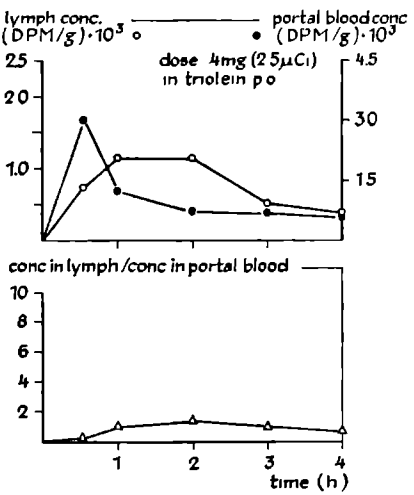
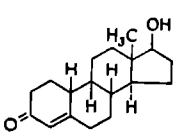


Fig 31
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of progesterone and the corresponding quotients of these concentrations
For further details see legend of fig 8

4.3.15. NANDROLONE



The results of the animal experiment of the first type are shown in fig. 32 and table 23.

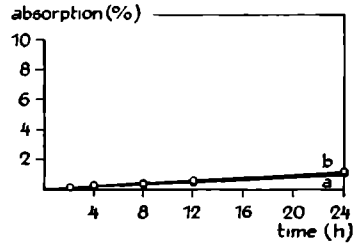


Fig 32
Cumulative absorption of nandrolone in thoracic duct lymph
For further details see legend of fig 9

Table 23. Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}C-nandrolone.

rat	dosage form	administered amount (mg)	administered act (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat
a	triolein	0 440	1	48 7	17 1	0 7	0 8	0 01	0 02
b	mic sol	0 440	1	54 9	15 0	0 8	0 5	0 01	0 02

The results of the animal experiment of the second type are shown in fig. 33.
The ΔR_m value in respect of butter yellow was found to be -0 39 (s d. 0.04) Detection was carried out using $KMnO_4$ spraying.

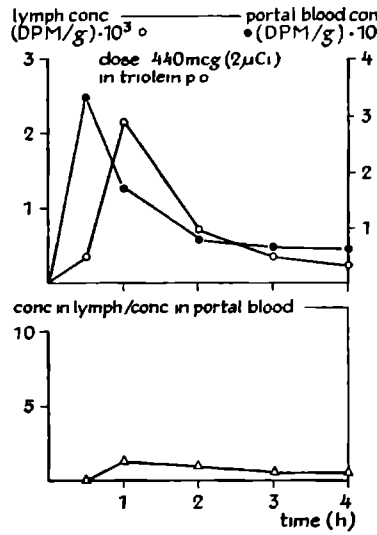
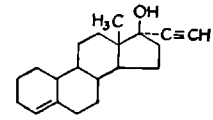


Fig 33
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of nandrolone and the corresponding quotients of these concentrations
For further details see legend of fig 8



4.3.16. LYNESTRENOL

The results of the animal experiment of the first type are shown in fig. 34 and table 24.

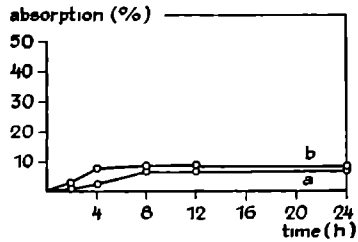


Fig 34
Cumulative absorption of lynestrenol in thoracic duct lymph
For further details see legend of fig 9

Table 24. Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}C-lynestrenol.

rat	dosage form	administered amount (mg)	administered act (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat
a	mic eol	3 5	0 5	54 1	11 1	3 2	0 5	< 0 001	0 04
b	trioleln	3 5	0 5	26 4	40 7	6 2	1 2	0 11	0 04

The results of the animal experiment of the second type are shown in fig. 35.
The ΔR_m value in respect of butter yellow was found to be 0.44 (s.d. 0.05). Detection was carried out using $KMnO_4$ spraying.

Discussion:
The ΔR_m value of lynestrenol in respect of butter yellow is relatively high. Therefore we find a larger lymphatic transport compared to the previous, much less lipophilic, steroids nandrolone and progesterone.

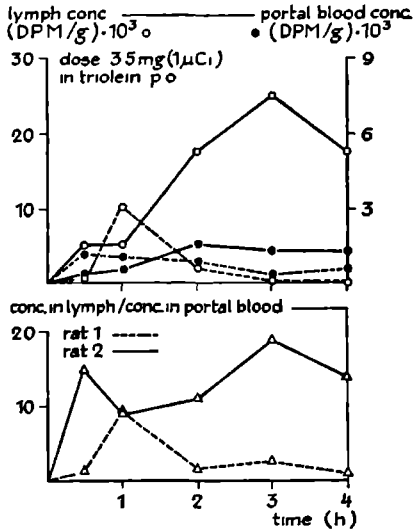
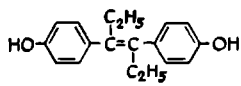


Fig 35
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of lynestrenol and the corresponding quotients of these concentrations
For further details see legend of fig 8



4.3.17 DIETHYLSTILBESTROL

The results of the animal experiment of the first type are shown in table 25
For rat a (table 25) the percentage of the apparent absorption, that was absorbed via the lymph tract, was from 0-12 hr after administration 0 49 per cent and from 12-24 hr after administration 0.01 per cent. So, in 24 hr this absorption was 0 50 per cent.
For rat b (table 25) these percentages were from 0-12 hr 0 40 per cent, and from 12-24 hr 0 10 per cent So, in 24 hr this absorption was 0 50 per cent.

Table 25 Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}C-diethylstilbestrol.

rat	dosage form	administered amount (mg)	administered act (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat
a	mic sol	0 100	1	45 4	1 0	0 27	1 2	< 0 001	< 0 001
b	triolein	0 100	1	57 3	1 8	0 21	1 2	< 0 001	< 0 001

The results of the animal experiment of the second type are shown in fig. 36.
The ΔR_m value in respect of butter yellow was found to be -0 07 (s d. 0.03). Detection was carried out using $KMnO_4$ spraying

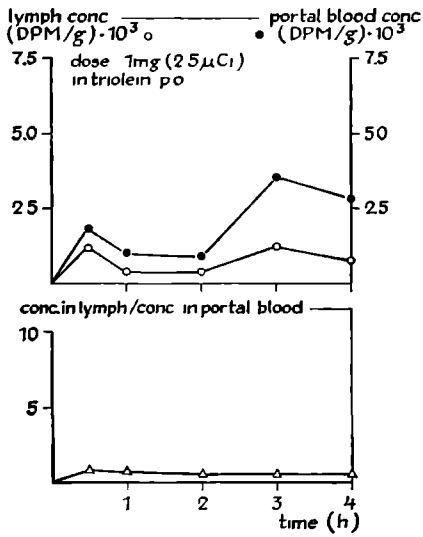
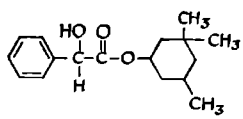


Fig 36
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of diethylstilbestrol and the corresponding quotients of these concentrations
For further details see legend of fig 8

4.3.18. CYCLANDELATE



The results of the animal experiment of the first type are shown in fig. 37 and table 26

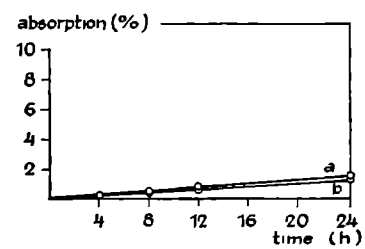


Fig 37
Cumulative absorption of cyclandelate in thoracic duct lymph
For further details see legend of fig 9

Table 26. Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}C-cyclandelate.

rat	dosage form	administered amount (mg)	administered act. (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat.
a	mic. sol.	2	0.5	1.2	79 3	1.6	0.07	0.003	0.04
b	triolein	2	0.5	0.5	64.0	1.3	0.8	< 0.001	0.03

The results of the animal experiment of the second type are shown in fig. 38.

The percentage of unconverted pharmacon 3-4 hr following oral administration and ½-1 hr following administration into the tail vein is shown in table 27.

The ΔR_m value in respect of butter yellow was found to be 0.18 (s.d. 0.04). Detection was carried out using $KMnO_4$ spraying.

Discussion:

The ΔR_m value of this ester, 0.18, is lower than that of dieldrin (0.24) or quinestrol. Cyclandelate appears to be saponified in lymph

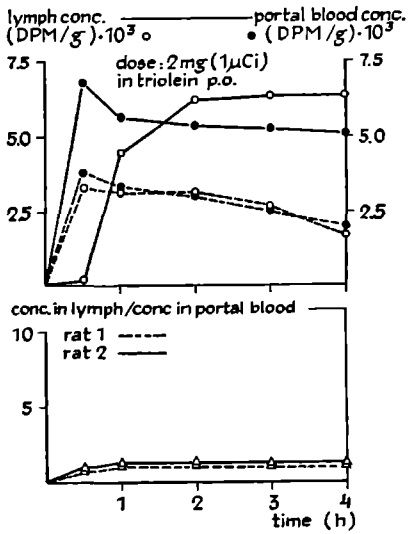
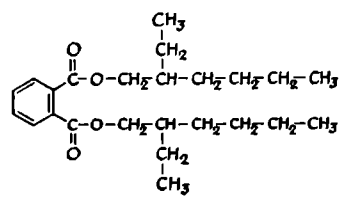


Fig. 38
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of cyclandelate and the corrsponding quotients of these concentrations.
For further details see legend of fig. 8.

Table 27. The presence of unmetabolized cyclandelate in mesenteric lymph and portal blood at 3-4 hr after oral administration (at ½-1 hr in case of i.v. administration).The values are presented as percentages of total ^{14}C radioactivity.

rat	dosage form	route of administration	unmetabolized pharmacon (%)	
			lymph	portal blood
1	triolein	p.o.	26	10
2	trioleln	p.o.	34	9

and even more so in the blood. Therefore an experiment was carried out to determine whether the saponification had already occurred to a considerable extent in the intestine. Cyclandelate appears not to be converted to any great extent in the intestinal contents. Approx. 20 per cent of the initial amount appeared to be hydrolyzed following 1 hr incubation in intestinal fluid of the rat.



4.3.19. DIOCTYLPHTHALATE

The results of the animal experiment of the first type are shown in fig. 39 and table 28.

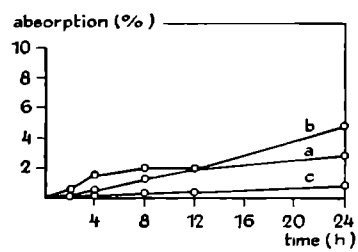


Fig. 39
Cumulative absorption of dioctylphthalate in thoracic duct lymph.
For further details see legend of fig. 9.

The results of the animal experiment of the second type are shown in fig. 40.

Table 28 Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}C-dioctylphthalate.

rat	dosage form	administered amount (mg)	administered act (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat
a	triolein	1	1	33 0	42 2	1 9	2 2	—	0 02
b	triolein	1	1	33 5	22 3	3 2	2 1	0 02	0 04
c	triolein	1	1	11 9	25 8	0 8	1 6	—	0 01

The percentage of unconverted product 3-4 hr following oral administration and ½-1 hr following administration into the tail vein is shown in table 29.
The ΔR_m value in respect of butter yellow was found to be > 1.23 by extrapolation. Detection was carried out using UV absorption.

Discussion:
D.O.P. is much used as a softener in plastics. Blood that was stored in plastic infusion bottles appeared to extract D.O.P. from the plastic (Jaeger and Rubin, 1970) Thus D.O.P. appeared in the infused body

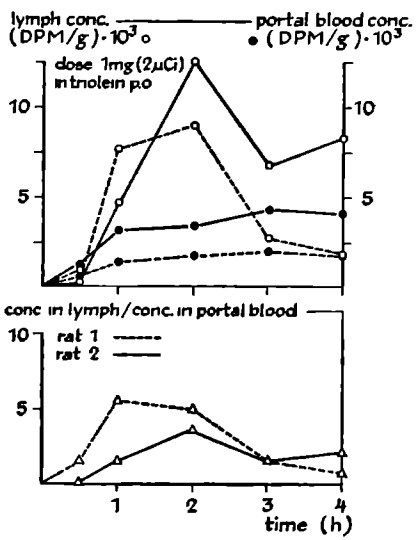


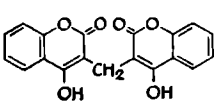
Fig. 40
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of dioctylphthalate and the corresponding quotients of these concentrations
For further details see legend of fig 8

Table 29. The presence of unmetabolized dioctylphthalate in mesenteric lymph and portal blood at 3-4 hr after oral administration (at ½-1 hr in case of i.v. administration). The values are presented as percentages of total ^{14}C radioactivity.

rat	dosage form	route of administration	unmetabolized pharmacon (%)	
			lymph	portal blood
1	triolein	p.o.	65	21
2	prop. glyc.	p.o.	78	23

and the unconverted product was demonstrated by gas chromatography. The ΔR_{in} value in respect of butter yellow appears to be large. This ester, though, appears already to be markedly saponified in the intestine. Incubation of D.O.P. for 1 hr with intestinal fluid produced a saponification of 79 per cent. The lymphatic absorption appeared to be small and the lymphatic/portal blood conc. quotient was also low. Finally the perirenal fat appeared to absorb only a small amount of radioactivity and the excretion via the urine appeared to be considerable, which one might expect from a hydrophilic metabolite. In conclusion it can be said that following oral administration of D.O.P. there is less danger of absorption of the product in the body than following parenteral administration. Also for this material the percentage of metabolite appears to be larger in portal vein blood than in lymph.

4.3.20. DICUMAROL



The results of the animal experiment of the first type are shown in fig. 41 and table 30.

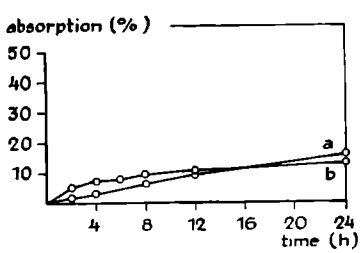


Fig. 41
Cumulative absorption of dicumarol in thoracic duct lymph.
For further details see legend of fig. 9.

Table 30. Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}C-dicumarol.

rat	dosage form	administered amount (mg)	administered act. (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat.
a	mic. sol.	1	1.5	22.4	12.7	12.4	5.3	0.3	0.1
b	mic. sol.	1	1.5	10.0	10.4	11.6	2.6	0.2	0.1

The results of the animal experiment of the second type are shown in fig. 42.
The ΔR_m value in respect of butter yellow was found to be < -0.39 by extrapolation. Detection was carried out using $KMnO_4$ spraying.

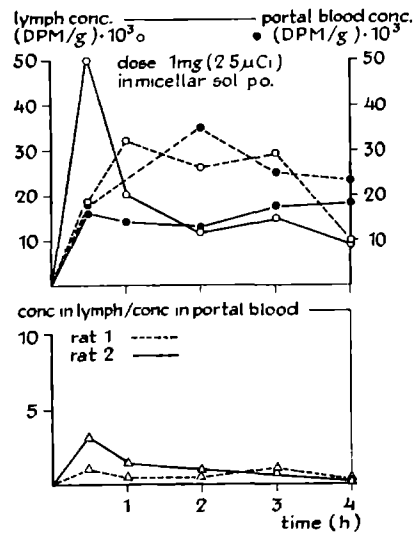
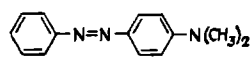


Fig. 42
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of dicumarol and the corresponding quotients of these concentrations.
For further details see legend of fig 8.

Discussion:
Dicumarol has a very low ΔR_m value, moreover this compound is practically insoluble in water. It has a large protein binding capacity. The lymphatic/portal blood conc. quotient appeared to be practically 1. Still the lymphatic transport of dicumarol and its metabolites continues regularly up to 24 hr following administration. The concentration in

cardiac blood appears to be considerable. Probably a great part of the dicumarol, transported via the lymphatic tract, is extracted from the blood vessels by equilibrium exchange between the blood and the tissues. So this lymphatic transport of dicumarol does not represent the direct absorption out of the intestine to the lymph vessels.

4.3.21. p - DIMETHYLAMINOAZOBENZENE = BUTTER YELLOW



The results of the animal experiment of the first type are shown in fig. 43 and table 31.

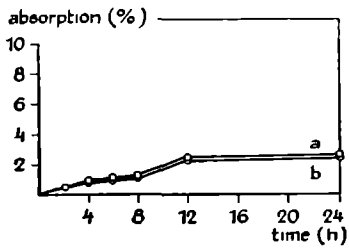


Fig 43
Cumulative absorption of p-dimethylaminoazobenzene in thoracic duct lymph
For further details see legend of fig 9

Table 31. Radioactivity measured in several biological fluids and tissues after oral administration of ^{14}-p-dimethylaminoazobenzene.

rat	dosage form	administered amount (mg)	administered act (μCi)	percentage of the administered amount of radioactivity for 24 hr in faeces and gut	for 24 hr in urine	for 24 hr in lymph	after 24 hr in total liver	after 24 hr in 1 g cardiac blood	after 24 hr in 1 g perirenal fat
a	triolein	2	0 5	8 0	72 4	2 5	1 0	0 1	0 03
b	mic sol	2	0 5	12 3	80 5	2.2	0 5	0 1	0 01

The results of the animal experiment of the second type are shown in fig. 44.
The ΔR_m value in respect of butter yellow is zero by definition. Detection was by visual examination since the compound is coloured.

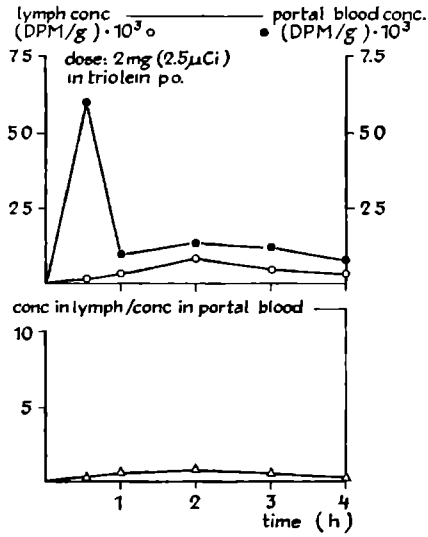


Fig. 44
Concentrations of radioactive compound in mesenteric lymph and portal blood at various times after administration of p-dimethylaminoazobenzene and the corresponding quotients of these concentrations.
For further details see legend of fig. 8

4.4. CONCLUDING REMARKS

The experimental results, as described above, clearly demonstrate that there is a selective lymph absorption of various compounds including some insecticides, halogenated hydrocarbons, a carcinogen such as methylcholanthrene and other pharmaca. This lymphatic transport particularly occurs when these compounds are administered in a fatty dosage form (chapter 3). This selective absorption can also be inferred from the great concentration of these pharmaca in the lymph fluid, at the peak of the absorption process, compared with the concentration in the portal blood. So, for instance, the concentration quotients (lymph/blood) for compounds which show an apparent lymphatic absorption during 24 hr of 10-20 per cent, are in the range of 30-100; however for compounds absorbed for more than 20 per cent via the lymph these quotients amount to 100-400. This is in good agreement with the difference in flow rate between lymph and portal blood as already mentioned in chapter 1.
Dicumarol is in this respect an exception. Although the amount of dicumarol measured in the 24 hr lymph is considerable, namely 13-16 per cent of the total amount absorbed from the gut, the lymph/blood concentration ratio appears to be only about 1. It must also be remarked, that the lymphatic transport of dicumarol gradually increases

during 24 hr (see fig. 41) in contrast to the other compounds studied, which show a maximum in their transport after 1-4 hr. For dicumarol the possibility exists that as well as a direct absorption via the lymph vessels, this drug also appears in the lymph via a redistribution from the blood.

As to the question posed in the introduction (4.1), whether there is any relationship between the lymphatic transport of a pharmacon and its lipid solubility, it is striking that only those compounds with a high lipophilicity are absorbed via the lymph tract to any considerable extent. For the different compounds investigated, the degree of correlation between lymphatic transport and lipophilicity was studied quantitatively. As is shown in fig. 45, it appears that there exists a

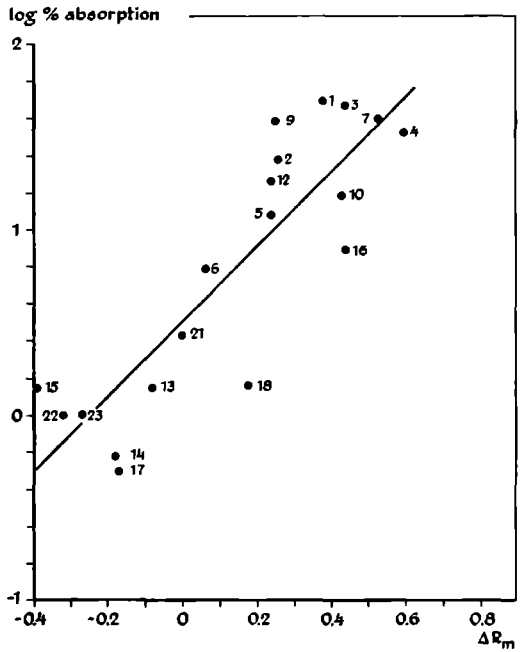


Fig. 45

The correlation between the apparent lymphatic absorption and lipid solubility. The apparent lymphatic absorption is presented as the percentage of the amount absorbed during 24 hr from the gut. The lipid solubility of the pharmaca is expressed as a ΔR_m value relative to butter yellow (4.2.4).

(1) DDT, (2) DDD, (3) DDE, (4) aldrin, (5) dieldrin, (6) lindane, (7) hexachlorbiphenyl, (9) methylcholanthrene, (10) Δ 1-THC, (12) quinestrol, (13) mestranol, (14) progesterone, (15) nandrolone, (16)lynestrenol, (17) diethylstilboestrol, (18) cyclandelate, (21) p-dimethylaminoazobenzene = butter yellow, (22) testosterone, (23) methyltestosterone.

linear correlation between the log of the apparent lymphatic absorption and the ΔR_m values of the different pharmaca relative to butter yellow. The relationship can be characterized by the following equation

$$\log \% \text{ absorption} = 2.01\ (\pm 0.63)\ \Delta R_m + 0.50\ (\pm 0.20)$$

$n = 19 \qquad r = 0.850 \qquad s = 0.380$

where r is the correlation coefficient
s represents the standard deviation
n refers to the number of different compounds tested.
In the equation the 95 per cent confidence intervals are given by the figures in parentheses

From the data given above it appears that there is a reasonable correlation Within a limited range of strong lipophilicity it is permissible to conclude that greater lipophilicity results in a greater lymphatic transport. This is in contrast to the conclusion of Levine (1969), that the degree of lipid solubility may only determine the degree of total absorption from the intestine but not the proportion of absorbed material leaving via the lymph. The conclusion of Levine, however, was only based on studies with quaternary ammonium compounds, which are less lipid soluble than the pharmaca used in our study
Nevertheless, on the basis of the results of the present correlation study, it cannot be excluded that, as well as the hydrophobic character of the compounds, other properties (electronic, stereochemical) also play a role in lymphatic absorption In chapter 5 it will be demonstrated that the binding of various lipophilic pharmaca in the chylomicrons and other lipoproteins is much greater after oral administration than during in vitro incubation with these lymph constituents Hence, it may be concluded that although lipid solubility plays a major role, there may be something beyond lipid solubility determining the incorporation of the pharmacon into the chylomicron and, as a result, influencing lymphatic transport This is consistent with the report of Cohn and Sieber (1970) regarding the transport of DDT in the rat

CHAPTER 5

BINDING OF VITAMIN D_3, DDT, DIELDRIN, QUINESTROL AND MESTRANOL TO THORACIC DUCT LYMPH COMPONENTS UNDER VARIOUS CONDITIONS

5.1. INTRODUCTION

It has been well demonstrated that a number of pharmaca are absorbed to a considerable extent via the lymph tract after oral administration in fatty vehicles. These pharmaca all have a strongly lipophilic nature. The ΔR_m value with respect to butter yellow of these compounds appears to be always > 0.2 (see chapter 4). Strong binding to chylomicrons is known for a number of them. DDT, (Cohn and Sieber, 1970), cholesterol (Treadwell and Vahouny, 1968), vitamin A and β carotene (Huang and Goodman, 1965), vitamin D (Schachter et al, 1964), vitamin E (Blomstrand and Forsgren, 1968b) and vitamin K_1 (Blomstrand and Forsgren, 1968a) appear to be present in the chylomicrons for 80-90 per cent of the quantity absorbed via lymph. The transport of these pharmaca in the chylomicrons may be based upon one or more of the following processes:

1. A non-specific form of interaction between pharmacon and fat components of the lymph like the chylomicrons, due to the marked lipophilicity of the pharmacon.

2. An interaction between the pharmacon and specific parts of the chylomicron. Namely in those chylomicrons consisting of a nucleus and a membrane. They consist of 80 per cent triglyceride, 11 per cent cholesterol (of which 80 per cent is esterified), 7 per cent phospholipids and 2 per cent proteins (see table 32) (Houtsmuller, 1969). The unstructured nucleus contains 99 per cent of the triglyceride, the esterified cholesterol and 30 per cent of the unesterified cholesterol (Zilversmit, 1965; Huang and Kuksis, 1967). The structured membrane consists of a mosaic of the protein, 70 per cent of the unesterified cholesterol and

Table 32. Properties of lymph fractions**.

lipoproteins* class and proteins	density range (g/ml)	flotation (s_f)	electrophoresis fraction	composition in percents					
				triglyceride	cholesterol	phospholipid	protein	N terminal aminoacids	
								major	minor
chylomicrons	0.95	400	——	80	11	7	2	ser thr	glu asp
VLDL	0.95—1.006	20-400	pre β lipoprotein	55	13	22	10	ser thr	glu asp
LDL	1.006—1.063	0-20	β lipoprotein	12	45	22	21	glu	ser thr
HDL	1.063—1.21	—	α lipoprotein	6	18	26	50	asp	
proteins	> 1.21	—	serumproteins	—	—	—	100		

* VLDL = "very low density lipoprotein".
LDL = "low density lipoprotein".
HDL = "high density lopoprotein".

** Data obtained from Houtsmuller (1969).

1 per cent of the triglyceride in a directed monolayer of phospholipids (Fraser et al, 1968; Lossow et al, 1969; Salpeter and Zilversmit, 1968). In spite of the fact that the chylomicron membrane is a monolayer, there remains a certain resemblance to the unit – membrane of Robertson (1957), which gives the best indication of the universal structure of all biological membranes up to now. The chylomicron shell, as well as the unit-membrane, contains a directed layer of lipids. The inner region of the unit-membrane is composed of a bimolecular layer of lipid molecules, particularly phospholipids and cholesterol.
Much work has been carried out on the binding of pharmaca to biological membranes. The idea is, that specific ability for binding to certain membranes might be the basis for the specificity observed in many of the biological actions of a number of pharmaca. Investigations according to this idea are reported for vitamin A, E and K_1 (Lucy and Dingle, 1964), vitamin D (Spirichev and Blazeheevich, 1968), steroids like stilbestrol and progesterone (Weissmann and Keiser, 1965), DDT (Tinsley et al, 1971), 3-methylcholanthrene (Weiner et al, 1971) and Δ l-tetrahydrocannabinol (Chari-Bitron, 1971).
Based on the discussed analogy between the chylomicron shell and biological membranes, one might also expect a specific interaction between the discussed pharmaca and the chylomicron shell.

3. Interaction between certain pharmaca and chylomicrons could be related to the process of chylomicron formation in the mucosa cells. One may imagine two possible processes: a. The pharmaca could be built into the chylomicrons by an active process. b. During the process of chylomicron formation, the pharmaca and the fat components of the chylomicrons are present in the mucosa cells in relatively high concentrations. The pharmaca could interact with fat components and together they could complete the formation of the chylomicrons. In this way the pharmaca may be captured within the shell of the chylomicrons. In respect of this the question arises as to whether the route of administration, e.g. oral or parenteral, plays a role in the degree of lymphatic transport.

As has been mentioned previously in this introduction, cholesterol, fat soluble vitamins and DDT are not bound to chylomicrons for 100 per cent, but only for 80-90 per cent. In relation to this it is of importance that recent investigations have shown that mucosa cells not only form chylomicrons but also other lipoproteins such as very low density

lipoproteins (VLDL) (Ockner et al, 1969), low density lipoproteins (LDL) and high density lipoproteins (HDL) (Roheim et al, 1966; Dietschy and Siperstein, 1965; Windmueller and Spaeth, 1972). It is also known that these lipoproteins and other proteins are present in mesenteric lymph in considerable amounts (Yoffey and Courtice, 1970). The question now arises, as to whether the latter mentioned lipoproteins and the proteins, are responsible for binding the 10-20 per cent of cholesterol etc. that is not bound by the chylomicrons.

We have tried to answer these questions by the following experiments: Five pharmaca were investigated: vitamin D_3, DDT, dieldrin, quinestrol and mestranol. These pharmaca are different in respect to their lymphatic transport after oral administration. The amount of lymphatic transport may be classified as: a. **considerable** for vitamin D_3 and DDT; b. **moderate** for dieldrin and quinestrol; c. **practically no transport** for mestranol (see table 33).

Table 33. Classification of some pharmaca in relation to their lymphatic transport after oral administration.

pharmacon	ΔR_m value in respect of butter yellow	lymphatic transport* in %	category
DDT	0 38 (s.d. 0.01)	50.2	considerable
vitamin D_3	1 23 (s.d. 0.12)	75-100	
dieldrin	0 24 (s.d. 0.01)	12.0	moderate
quinestrol	0.24 (s.d. 0.03)	18.3	
mestranol	-0.08 (s.d. 0.06)	1.6	low

* The amount of pharmacon, transported via the thoracic duct for 24 hr following administration is given as percentage of the apparent absorption. The average amounts of 3-8 experiments are given. Data about the lymphatic transport of vitamin D_3 were obtained from literature (see chapter 4).

Binding of these pharmaca to lymph components was obtained in three ways:

- Lymph, obtained after fat administration (i.e. control lymph) was incubated in vitro with the pharmaca.
- The pharmacon, in a fatty dosage form, was administered into the

stomach of the rat and after this thoracic duct lymph was sampled.

- The pharmaca were injected intravenously into the tail of the rat and after this, thoracic duct lymph was sampled. Two hours before the injection, triolein was administered into the stomach of the rat so that at the moment of the injection a considerable amount of chylomicrons and other lipoproteins were present in the thoracic duct lymph.

The amounts of pharmacon that were bound to chylomicrons, VLDL, LDL together with HDL and to the other proteins were investigated. The amounts of these pharmaca that were bound to chylomicron core and shell were also studied.

5.2. EXPERIMENTAL PROCEDURES

5.2.1. In vivo and in vitro experiments.

The binding of the five pharmaca to the lymph fractions was accomplished in three ways.

A. In vitro incubation of control lymph with the pharmaca. The rats were cannulated and treated according to the first type of animal experiments (see chapter 2). Lymph was collected after repeated administration of 0.3 ml triolein. 8 ml portions of this lymph were incubated in duplicate with the different pharmaca. The pharmacon (see table 34) was added to the lymph, dissolved in 0.1 ml ethanol. The incubation was carried out at 37 °C for 8 hr under continuous shaking (Bell and Schwartz, 1971).

Table 34. Concentration and activity of the investigated pharmaca in thoracic duct lymph.

radioactive pharmaca	specific activity in μCi/mg	amount in μg per 8 ml lymph
^{14}C-DDT	2.5	50
^{14}C-vitamin D_3	83.0	1
^{14}C-dieldrin	2.5	50
^{3}H-quinestrol	500.0	1
^{14}C-mestranol	82.5	1

B. Administration of the pharmaca into the stomach.
Rats were cannulated and treated according to the first type of animal experiments. The thoracic duct lymph was collected from 2 hr until 8 hr after administration of the pharmaca into 0.3 ml triolein. Portions of 8 ml of this lymph were investigated. The quantity of the pharmacon administered to the rat was chosen such that the quantities of pharmacon mentioned in table 34 were again present in the lymph samples.

C. Administration of the pharmaca in the tail vein.
The animal experiment carried out was also of the first type. 1½ hr before the injection of the pharmacon into the rats 0.3 ml triolein was administered via the stomach cannula. At the moment of injection of the pharmacon there was a considerable amount of lipoproteins present in the lymph. The pharmacon, dissolved in 150 mg propylene glycol, was injected into the tail vein. The thoracic duct lymph was collected for 6 hr following the injection. Lymph portions of 8 ml were analyzed. The quantity of pharmacon in these lymphs was the same as described in table 34.

5.2.2. Analytical procedures.
By ultracentrifugation the lymph was divided into 4 fractions: chylomicrons, VLDL, LDL-HDL and other proteins. A preliminary test of these fractions was carried out on control lymph by subjecting the lymph fractions to electrophoresis. It was determined previously, by means of dialysis, that the pharmaca were bound to the fractions for more than 99 per cent. The content of chylomicrons and VLDL in these fractions was determined by estimating the amounts of triglyceride in these fractions; the fractions LDL-HDL and other proteins were determined by estimating the protein content. It was assumed that the lipoprotein composition was as is reported by Houtsmuller (1969) (table 32). By means of the amounts of triglyceride and proteins determined, the percentage of lipoprotein could be calculated. The protein content of the fraction LDL-HDL was supposed to be 35.5 per cent of total weight. The amount of pharmacon in the lymph fractions was measured by counting the radioactivity in a liquid scintillation counter according to the method described in chapter 2.
The chylomicrons were separated further into core and membrane. The oil layer of the cores which developed was separated from the membranes by centrifugation. The amount of pharmacon in the core and the membrane was measured by counting the radioactivity. All lymph

samples and lymph fractions were stored at 4 °C and analyzed within 3 days.

5.2.2.1. Dialysis.
Dialysis was carried out in Visking, seamless cellulose tubing of 8/32". Duplicates of radioactive DDT, mestranol, dieldrin, vitamin D_3 and quinestrol in quantities of a few micrograms were added to portions of 1 ml control lymph. This was dialysed against physiological saline for 17 hr at 20 °C. DDT, dieldrin, vitamin D_3 and quinestrol appeared to be bound to the lymph fractions for $>$ 99.9 per cent, whilst mestranol was bound for 99.0 per cent.

5.2.2.2. Separation of lymph in fractions by means of ultracentrifugation.
The blood cells were always separated from the lymph beforehand by centrifuging for 10 min at 2000 RPM. In each experiment this cell-free lymph was separated into 4 fractions. The course of the fractionation was as follows:

A. *Chylomicron fraction.*
This was obtained by placing a liquid column of 0.85 per cent NaCl solution (Lascelles and Wadsworth, 1971) above the lymph sample. After centrifugation for 1 hr at 5 °C in a M.S.E. Refrigerator Centrifuge at 20,000 g the chylomicron fraction could be removed with a pasteur pipette.

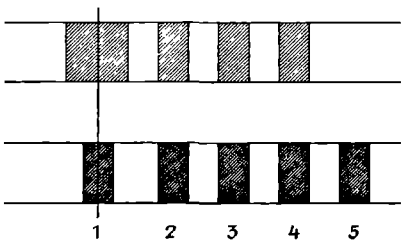
B. VLDL fraction.
This fraction was obtained by centrifuging the remaining lymph for 18 hr at 120,000 g in an Omega II Christ Ultracentrifuge (Viikari, 1969). Then the fraction was removed with a pasteur pipette.

C. LDL-HDL.
The remaining liquid was brought to a specific density of 1.21 with a concentrated solution of saline. It was then centrifuged for 18 hr at 120,000 g. The LDL-HDL fraction was removed with a pasteur pipette. (Havel et al, 1955).

D. Protein fraction.
This remained on the bottom of the centrifuge tube after the process described under C.

5.2.2.3. Purity test of lymph fractions.
The purity of the lymph fractions was tested by electrophoretic procedures.

Electrophoresis of control lymph on cellulose acetate gave electropherograms after fat and protein staining as illustrated in fig. 46 (Sargent, 1969; Jansen, 1971). The electrophoresis was carried out on cellulose acetate strips of 160 x 25 mm (Schleicher und Schüll) using the "Elphor" apparatus (Bender and Hobein). The procedure was carried out according to Perl and Voggel (1969). The samples were placed on the strips with a special applicator (Jansen, 1971). This was made of V_2H steel, length 2 cm, width 0.5 mm, thread distance 100 μ.



1. chylomicrons
2. β lipoprotein
3. pre β lipoprotein
4. α-lipoprotein
5. —

1. γ globulin
2. β globulin
3. α_2 globulin
4. α_1 globulin
5. albumin

Fig. 46
Electropherogram obtained after fat and protein staining.

After the developed electropherograms had been stained for protein with amido black 10 B or for fat with Schiff's reagent they were dried by means of a cold air current and made transparant with liquid paraffin. With the "Elphor-Integraph" an extinction curve was constructed for these electropherograms. A planimeter was used to calculate the relative percentages of lipoproteins and other proteins from these curves. This method was tested with a solution of albumin (Poviet) and globulin (Cohn fraction II from Koch-Light) in quantities approximating to those found in thoracic duct lymph (Yoffey and Courtice, 1970), namely 1000 mg albumin and 250 mg globulin in 25 ml physiological saline.
Control lymph was separated into the 4 fractions by ultracentrifugation in the manner described. Electropherograms were made from the obtained fractions. By means of the procedures described the extent of separation of the lymph fractions was tested. It appeared that the electropherograms of each separated fraction corresponded fairly well with the expected electrophoretic pattern, although contaminations from other fractions were observed (1-5 per cent).

5.2.2.4. Triglyceride determination.
The triglyceride content of the chylomicron and the VLDL fraction was determined according to the method of Eggstein and Kreutz (1966),

using a Biochemica Test Combination from Boehringer. The procedure is based on an enzymatic determination of glycerol which was obtained by saponification of the fat in a sample. With a spectrophotometer (Carl Zeiss M_4Q III with photomultiplier PMQ II) the course of the enzymatic reaction could be followed at 340 nm.

5.2.2.5. Protein determination.
This was carried out with the Folin phenol reagent according to Oliver (1951) as used by Faulkner and King (1970) for protein determination in blood serum.

5.2.2.6. Separation of chylomicrons into core and shell.
This was carried out according to the procedure of Zilversmit (1965). The chylomicron fractions were kept at - 40 °C for 12 hr. Then the fractions were warmed for ½ hr to + 40 °C. This process was repeated 5 times. In this way a separation was achieved between nuclear material and outer shell material. The fractions could be separated by centrifugation. The composition of both fractions has already been described in the introduction. The mass of the membrane material appeared to average 7.5 per cent of the chylomicron (Zilversmit, 1965; Huang and Kuksis, 1967).

5.3. RESULTS AND DISCUSSION

Since from the dialysis experiments it appears that the investigated pharmaca are bound to the lymph fractions for more than 99 per cent, it can be assumed that the lymph fractions that are obtained after separation by centrifugation, indeed contain the pharmaca in a bound state.
The extent of binding of the pharmaca to the lymph fractions and the quantity of these fractions is shown in table 35 A. It is immediately apparent that the greatest percentage of the pharmacon is practically always bound to the chylomicron fraction, both when the pharmacon binding to the lymph fractions is obtained via oral or i.v. administration, or when the pharmacon was incubated in vitro with control lymph. The chylomicron fraction is usually present in the greatest quantity, whereas the protein fraction is also present in relatively great quantities. The specific binding capacity of this fraction is relatively small. Table 35 B shows this more clearly. This table was derived from table 35 A by calculation. The binding percentages of the pharmaca were

divided by the number of mg of the fraction and the figures obtained were converted to ratios, relative to the protein fraction taken as unity. The binding of the pharmaca to the chylomicron fractions, independent of how the binding of the pharmaca to the lymph fractions has been brought about, usually appears to be relatively larger than the binding to the protein fraction. Vitamin D_3 following i.v. administration is an exception. This is a special case and will be subsequently discussed. The fact that also following incubation and i.v. administration the binding of the pharmaca to the chylomicrons is so great, seems to confirm the impression that the degree of lipophilicity is primarily responsible for the binding of the pharmaca to the chylomicrons and that specific interactions capable of occurring in the mucosa cells, especially following oral administration, are only of secondary importance. It has to be taken into account here that the results found are obtained by radioactivity measurements without correcting for the radioactivity of metabolites formed. An impression of the extent to which unmetabolized pharmacon can be present is given in table 36. This table was constructed from data from chapter 4. 3-4 hr following oral administration and ½-1 hr following i.v. administration of the pharmacon the percentage of unmetabolized pharmacon in mesenteric lymph and portal blood was determined.
Conversion of the pharmaca after in vitro incubation with these lymphs was found to be smaller than in the lymphs obtained following oral and i.v. administration. The percentage of metabolites usually appeared to be relatively small in comparison with e.g. the binding ratio between chylomicron and lipoprotein fractions, so that in general the unmeasured metabolites have little influence on the conclusions.
It appears from table 35 A that the amounts of the VLDL and LDL-HDL fractions are often considerably smaller than those of the chylomicron and protein fractions. The question, posed in the introduction as to the state of the 10-20 per cent of cholesterol, fat soluble vitamins and DDT in the lymph, that was not found in the chylomicron fraction after oral administration, can also be answered from table 35 A. Attention must be paid to the results following b, oral administration. Except for vitamin D_3, the greater part appears to be bound to the VLDL and LDL-HDL fractions. For vitamin D_3 and also for mestranol a large part appears to be bound to the protein fraction. Table 35 B shows that the binding ratios e.g. in respect of the VLDL fraction are still 6 and 11 for respectively vitamin D_3 and mestranol. A marked binding to the protein fraction could also be explained by the data from table 36. The metabo-

Table 35 A Binding of DDT, vitamin D_3, dieldrin, quinestrol and mestranol to

pharmacon	binding to the lymph fractions following***	chylomicrons		VLDL	
		binding* of pharm (%)	amount** of chylomicr (mg)	binding of pharm (%)	amount** of VLDL (mg)
DDT	a Incubation	87 1 84 5	45	9 3 10 0	12
	b oral administration	96 4 96 4	204	1 6 1 4	0 6
	c i v administration	72 0 70 0	77	8 0 8 0	3 0
vitamin D_3	a Incubation	87 2	100	3 3	5 5
	b oral administration	85 5 85 5	113	1 0 1 0	0 6
	c i v administration	8 0 6 0	76	 1 0	1 6
dieldrin	a Incubation	87 9 85 6	45	8 0 9 4	12
	b oral administration	97 2 96 8	200	1 7 1 7	1 4
	c i v administration	95 0 95 0	76	3 0 3 0	3 0
quinestrol	a Incubation	72 0 83 8	45	9 1 8 5	12
	b oral administration	72 1 74 1	47	7 1 7 5	1 5
	c i v administration	55 0 56 0	53	14 0 14 0	2 2
mestranol	a Incubation	93 0	107	2 0	5 5
	b oral administration	30 3 34 2	25	18 8 21 8	3 1
	c i v administration	55 0 54 0	75	5 0 5 0	2 8

lymph fractions

LDL-HDL		protein fraction	
binding of pharm (%)	amount** of LDL-HDL (mg)	binding of pharm (%)	amount** of protein fraction (mg)
26	17	10	30
43		12	
15	41	05	118
14		05	
90	12	110	33
100		120	
10	04	85	26
24	70	111	33
30		105	
150	20	770	33
110		820	
27	17	14	30
33		17	
08	15	03	114
10		05	
10	16	10	42
10		10	
100	17	89	30
22		55	
150	12	58	60
135		49	
140	22	170	22
140		160	
30	18	20	34
175	21	334	52
158		289	
100	41	300	42
80		330	

* The pharmaca binding to the lymph fractions is given as the radioactivity in the lymph fraction divided by the radioactivity in the total lymph sample (in per cents)

** The amounts of chylomicrons, VLDL, LDL-HDL and the protein fraction (in mg) derived from 8 ml lymph

*** The lymphs were obtained in 3 ways, namely after incubation of the pharmaca with thoracic duct lymph, accumulated after administration of triolein and after oral and intraveous administration of the pharmaca and accumulation of the thoracic duct lymph 2 hours before administration of the pharmacon, dissolved in 0 1 ml propylene glycol, into the tail vein, 0 3 ml of triolein was administered orally to these rats The oral administration was carried out with the pharmacon dissolved in 0 3 ml triolein

Table 35 B. Binding ratios of the pharmaca between the lymph fractions per unit of mass*.

pharmacon	binding to the lymph fraction by	binding ratio per unit mass			
		chylomicrons	VLDL	LDL-HDL	protein fraction
DDT	a. incubation	57	24	6	1
	b. oral admin.	125	625	87	1
	c. I.v. admin.	3	8	24	1
vit. D_3	a.	3	2	8	1
	b.	3	6	1	1
	c.	1/24	¼	3	1
dieldrin	a.	38	15	4	1
	b.	140	350	28	1
	c.	50	40	25	1
quinestrol	a.	7	3	2	1
	b.	16	55	130	1
	c.	1	8	8	1
mestranol	a.	15	7	30	1
	b.	2	11	13	1
	c.	1	2	2	1

* The figures were obtained by dividing the binding percentages from table 35 A by the mass of the fraction (mg) and then rounded off as ratios taking the protein fraction as unity.

lites formed (for mestranol approx. 30 per cent of the radioactivity measured in the lymph) are usually less lipophilic, and because of that a larger protein binding might be expected. The percentage of protein binding found would, in that case, not be due to unmetabolized vitamin D_3 and mestranol. Moreover, the lipophilicity of mestranol is so low, that based only on a small lymphatic transport therefore, a different binding form must be expected (see chapter 4).

It is of general importance to observe that the considerably lipophilic pharmaca are bound to the lipoproteins to a much greater extent than to the protein fraction. This is particularly obvious in table 35 B from the ratios per unit mass. Following oral administration there is, vitamin

D_3 excepted, a rough analogy to be seen between lipoprotein binding, in respect of protein binding, and lipophilicity. One can also see in table 35 B that except for vitamin D_3 and for mestranol – the latter product is considerably metabolized following oral and i.v. administration (see table 36) – the binding ratio to the chylomicron fraction obtained in lymph following oral administration, is greater than that obtained following incubation or i.v. administration.
This is even more obvious for the binding ratio of the VLDL fraction. It looks as though there is a special mechanism operative following oral administration through which these pharmaca are more strongly bound to the chylomicrons and the other lipoproteins. As has already been mentioned in the introduction, we can assume that the VLDL and

Table 36. The presence of unmetabolized pharmacon in mesenteric lymph and portal blood at 3-4 hours after oral administration and at ½-1 hour after i.v. administration.

pharmacon	mode of administration	percentage of unmetabolized pharmacon in	
		lymph	portal blood
DDT	p.o.*	97	72
		97	72
	i.v.**	80	83
vitamin D_3	p o.	90	27
	i.v.	86	57
dieldrin	p.o.	92	85
		98	85
	i.v.	96	51
quinestrol	p.o.	93	43
		83	43
	i.v.	81	45
mestranol	p.o.	70	14
		73	26
	i.v.	64	37

* The oral administration of the pharmaca was carried out in 0.3 ml triolein. This experiment was always conducted on 2 rats.

** 2 Hours before the administration of the pharmaca, dissolved in 0.1 ml propylene glycol, into the tail vein, 0.3 ml of triolein was orally administered to the rats. This experiment was always conducted on 1 rat.

the LDL-HDL fractions can also be formed in the mucosa cells. As far as the chylomicrons are concerned we see in table 38, that a greater relative binding ratio of the pharmaca occurs in the nucleus, analogous to the mentioned phenomenon following oral administration. In the lymph samples, obtained after incubation and i.v. administration, the shell of the chylomicron binds relatively more pharmacon than following oral administration, whereas these binding ratios per unit mass are even absolutely greater following the first mentioned mode of administration. It looks as if, following incubation and i.v. administration, the pharmacon binds itself to a larger extent to the shell, due to apposition with the outside of the completed chylomicron, whereas the pharmacon has access to the nucleus to a higher extent following oral administration. Without being able to decide from these experiments which interaction form between chylomicron and pharmacon plays a role following oral administration, besides the one based on lipophilicity, we conclude that this special interaction form, e.g the form mentioned in the introduction under 3b, in which it is assumed, that the pharmacon is caught within the shell of the chylomicron during the process of chylomicron formation, could be possible. Since the shell of the VLDL and the LDL-HDL particles contain considerably more constituents than the shell of the chylomicrons, this might explain the relatively greater binding with these lipoproteins following oral administration.
Dicumarol has little lipophilicity (see chapter 4) and a marked protein binding. It is practically insoluble in water. Table 37 shows that it is bound to the protein fraction to a considerable degree Table 38 shows that is binds particularly to the shell of the chylomicron following oral administration This shell contains among other things protein, so that the chylomicron can possibly generate binding of dicumarol via this

Table 37. Binding* of dicumarol to the lymph fractions following oral administration.

chylomicrons		VLDL		LDL-HDL		protein fraction	
DPM	per cent	DPM	per cent	DPM	per cent	DPM	per cent
4191	39	2600	24	1062	10	2859	27

* The binding of ^{14}C-dicumarol is mentioned as percentage of the found radioactivity in the lymph fraction, divided by the total quantity of radioactivity in the lymph sample

Table 38 Binding ratio of the pharmaca between shell and nucleus of the chylomicrons per unit mass shell and nucleus*.

pharmacon	binding to the lymph fraction following	binding ratio per unit mass**	
		shell	nucleus
DDT	a. incubation	12	7
	b oral admin	12	16
	c i v admin	12	7
vitamin D_3	a	12	4
	b	12	10
	c	12	9
dieldrin	a	12	6
	b	12	18
	c	12	15
quinestrol	a	12	2
	b	12	5
	c	12	4
mestranol	a	12	6
	b	12	17
	c	12	8
dicumarol	b	12	2

* The determinations were conducted using a sample of the chylomicrons obtained by the experiments mentioned in table 35 A

** In the determination of this ratio the mass ratio between shell and nucleus 7½% 92½% = 1 . 12 was taken into account.

5.4 CONCLUSION

The results of the experiments carried out agree with the concept that the interaction between strongly lipophilic pharmaca, such as DDT, and chylomicrons, primarily comes about as a function of the great lipophilicity of DDT. Besides that, there is possibly another form of interaction occurring following oral administration through which relatively more pharmacon appears in the nucleus of the chylomicron. It is difficult to decide from these experiments that a specific binding to the chylomicron shell, as discussed in the introduction, occurs. This is also true for vitamin D_3, which shows in table 35 B abnormal binding ratios as compared with DDT and dieldrin. The binding of vitamin D_3

to the protein fraction is relatively much greater than of the other pharmaca. Following i.v. administration this binding is even more pronounced with the protein fraction. Something special is occurring here, the explanation for which we may find in recent publications. Various investigators have found that vitamin D_3 in serum is, for the greater part, bound to proteins and not, or nearly not, to lipoproteins. Rikkers and DeLuca (1967) made observations at regular time intervals following oral and i.v. administration of vitamin D_3. Their observations suggest that the vitamin is transported to the liver by lipoproteins following administration. There a specific event occurs which results in the formation of a complex of the vitamin with a non-lipoprotein fraction, which then appears in the serum. Peterson (1971) described the isolation and characterization of a specific vitamin D-binding plasma protein. The results found by us can be explained in terms of these findings. As already mentioned above, dicumarol appears to be bound to a great extent to the protein fraction. In chapter 4 one can see that dicumarol, after oral administration, shows a concentration quotient of approx. 1 in lymph with respect to portal blood. So this pharmacon does not show a specific larger absorption via the lymph tract. Apparently strong binding to protein is not specifically responsible for great lymphatic transport, as opposed to the marked binding of for instance DDT to lipoproteins.

CHAPTER 6

INFLUENCE OF FATTY AND NON-FATTY DOSAGE FORM ON DISTRIBUTION AND METABOLISM OF DDT AND ALDRIN IN THE RAT

6.1. INTRODUCTION

When DDT is administered orally to the rat in micellar solution, about 50 per cent of the amount absorbed is transported in the lymph tract. On the other hand, when DDT is given in a non-fatty solution (propylene glycol) only about 14 per cent is transported in the lymph tract (see chapter 3). Also aldrin is absorbed in a greater amount via the lymph tract (about 33 per cent of the apparent absorption, see chapter 4) when given in triolein than when given in propylene glycol (about 10) per cent). It is believed that the absorption from the gut occurs via the lymph tract and the portal system (see 3.3.1.). So, if a great part of the absorbed DDT or aldrin is transported via the lymphatics, a small part has gone via the portal system. What are the consequences of absorption via the one or the other route as to the distribution and metabolism of DDT and aldrin?

Theoretically, if the pharmacon is absorbed exclusively via the portal system, then it goes directly to the liver (see fig. 47). The liver has a great capacity for metabolizing, eventually inactivating, a pharmacon. For instance, some steroids like testosterone and nandrolone can only be therapeutically effective when given as an injection, so that an initial passage through the liver is avoided (Biskind and Mark, 1939; Biskind, 1940; Kupperman, 1965).

If, on the other hand, the pharmacon is absorbed exclusively via the lymph tracts of the gut (the chyle vessels) then it will come into the blood via the vena subclavia and then it is possible that the pharmacon reaches its sites of action or that it is captured in certain tissues like the fat depots for instance, without passing the liver. The blood flow in

the splanchnic area of man in rest is about 1800 ml/min and his heart minute volume about 5250 ml (Rein und Schneider, 1960). This means that during one circulation of blood through the body about 1/3 passes the liver (see fig. 47). If a pharmacon, transported exclusively via the lymph tract into the blood of the vena subclavia, does not leave the vascular system, then it can be calculated that, after 6 circulations through the body, about 9 per cent of the pharmacon still has not passed the liver.

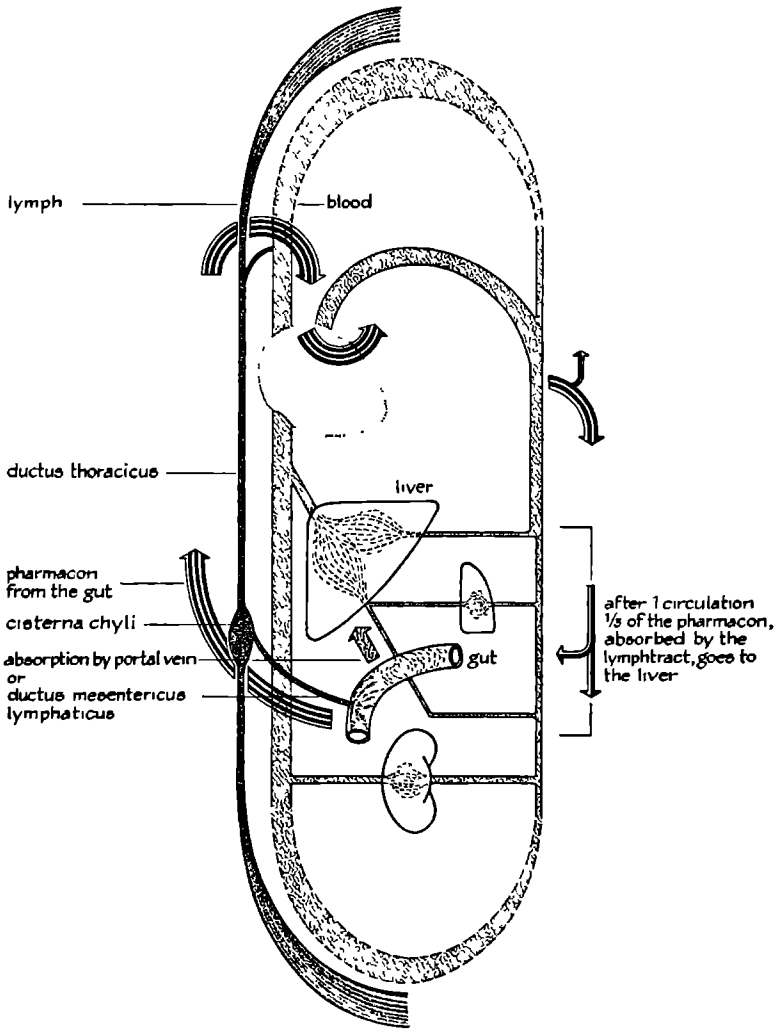


Fig 47

If an orally given pharmacon is absorbed exclusively via the lymphatic system (and is not changed during absorption) then it will be transported in the vascular system much the same as if it was administered parenterally.

6.1.1. Role of the chylomicrons in drug distribution.

The greater amount of the drug absorbed via the lymph tract compared to the portal system is not the only factor that could influence the pattern of distribution and metabolism of this drug. The fact that the lymphatic transport of pharmaca appears to be coupled to fat transport may also be of importance. In this respect the chylomicrons play a role, and to a much smaller degree so do the other lymph components, such as the lipoproteins and the proteins (see chapter 5).

If we suppose that the pharmaca remain present in the lymph components when these merge from the thoracic duct into the blood of the vena subclavia and after that are transported with this blood in the vascular system, then the distribution of these pharmaca will be particularly coupled to the fate of the chylomicrons. Some characteristic facts are now known about the distribution (and the metabolism) of the various fat components of the chylomicrons in the body.

If a pharmacon, present in the chylomicrons, shows affinity for one or more fat components present there, and follows these fat components in their distribution through the body, then the distribution of this pharmacon would be predictable based on our knowledge of the distribution of these fat components.

Chylomicrons are structured (see 5.1.). In the nucleus there are triglyceride and cholesterol esters. This nucleus is surrounded by a stabilizing membrane which is composed of a mosaic of protein, cholesterol and triglyceride (1 per cent) in a directed monolayer of phospholipids. The chylomicrons disappear out of the blood very rapidly. It is generally accepted, that during the first step of chylomicron metabolism about 80 per cent of triglyceride is removed by lipolysis. This action is mediated by lipoprotein lipase in the peripheral tissues, especially adipose tissues (Redgrave, 1970; Olivecrona and Fex, 1970; Bragdon and Gordon, 1958).

The apparent half life of chylomicron - triglyceride appeared to be 2.8 $\pm$ 0.37 (s.d.) min in unanesthetized rats (Harris and Felts, 1970). After lipolysis the residue of the chylomicron appears in the plasma as a high density lipoprotein, which is subsequently metabolized mainly

by the liver. So for instance cholesterol, a normal constituent of the chylomicrons, is found both esterified and free. It was reported by Olivecrona and Fex (1970), that this compound goes mainly to the liver. Vitamin D_3, which has an analogous sterol structure, is found to behave similarly to cholesterol in this respect. It was shown by Norman and DeLuca (1963), that ^{3}H-vitamin D_3 after oral administration to the rat is transported for the greater part via the lymphatics and rapidly accumulates in the liver. This demonstrates the general principle that the liver is avoided when a pharmacon is not absorbed via the portal system but via the lymphatics, does not hold true if the pharmacon is transported, like cholesterol, in the residual chylomicron after lipolysis. In that case a specific transport to the liver occurs.

6.1.2. Set-up of the present experiments.
For investigating the influence of fatty or non-fatty dosage form on the distribution and metabolism of a pharmacon that is absorbed in a considerable amount via the lymph tract, DDT and aldrin were chosen as examples.

6.1.2.1. DDT.
The choice of DDT in this study was advantageous, because much information about the lymphatic absorption of DDT has been gathered by us (see chapter 3, 4 and 5). For instance, it was found that DDT is captured for a great amount in the fat tissue of the body.
An impression of distribution and metabolism was obtained in the following ways: **A. Distribution of DDT and its metabolites between fat tissues and liver in relation to fatty or non-fatty dosage form.**
When DDT is absorbed via the portal system one could expect a different ratio of the amounts of DDT and its metabolites in liver and perirenal fat tissue than when DDT is absorbed via the lymph tract (see fig. 47). In order to check this, ^{14}C-DDT in fatty micellar solution or in non-fatty propylene glycol, was administered in the stomachs of rats. Several hours after administration a sample of perirenal fat and the liver were taken surgically, and ^{14}C radioactivity per g of tissue was determined. The quotients of radioactivity in 1 g fat / 1 g liver were calculated for each rat. These quotients, representing the distribution of radioactivity to fat and liver, were plotted as a function of time.
B. **Ratio between DDT and metabolites in perirenal fat in relation to fatty or non-fatty dosage form.**
DDT in primarily metabolized to DDD (see fig. 48) in the liver of man and rat (Morgan et al, 1971; Peterson and Robison, 1964). Thereafter

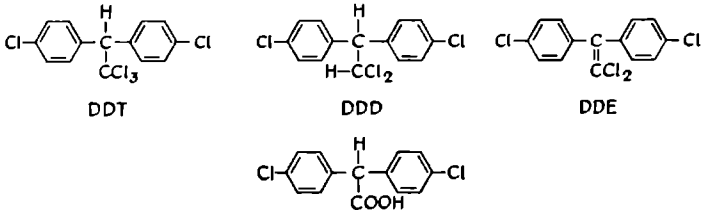


Fig 48

DDD is metabolized via various intermediates to DDA DDA is soluble in water. DDT and DDD are both found in fat tissue. DDT is metabolized poorly to DDE in man (Morgan and Clifford, 1971). This also holds true for the rat (Peterson and Robison, 1964) The metabolism of DDT to DDD occurs particularly in the liver In this respect, the intestinal flora of the rat only plays a minor role (Ottoboni and Ferguson, 1968, Alary et al, 1971) Therefore, when the absorption and subsequently the transport of DDT to the liver and perirenal fat is different in relation to fatty or non-fatty dosage form, one could also expect a different ratio of DDT to DDD in perirenal fat in relation to the dosage forms In that DDT, that is transported primarily via the portal tract to the liver, can be metabolized in the liver to DDD Thereafter unmetabolized DDT and DDD is redistributed from the liver to, for instance, perirenal fat On the other hand, when DDT is transported primarily via the lymph tract, then it may be stored in fat tissues as unmetabolized DDT. In the present experiments the amounts of ^{14}C-DDT and its metabolites ^{14}C-DDD and ^{14}C-DDE were determined in samples of perirenal fat, obtained 12 hr after administration in the stomach of ^{14}C-DDT in micellar solution or in propylene glycol respectively.

6 1 2 2 Aldrin

Aldrin was chosen for these experiments for the following reasons The general view is, that aldrin is metabolized very rapidly to its epoxide dieldrin (see fig 49) This occurs nearly exclusively in the liver (Hunter et al, 1960; Wong and Terriere, 1965). Aldrin as well as dieldrin

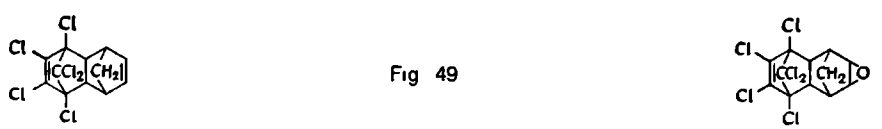


Fig 49

have a great fat-solubility and are taken up in a considerable amount in the retroperitoneal fat tissue (Heath and Vandekar, 1964). With aldrin the same experiments were done as described above for DDT.

6.2. EXPERIMENTAL PROCEDURES

6.2.1. Animal experiments

The rats were fasted 15-20 hr before the administration of the pharmacon. During this time and also during the experiment the rats had free access to water. The pharmacon was administered to the rats with a cannula via the mouth into the stomach. 1 mg DDT containing 1 μCi ^{14}C-DDT, was administered on the one hand in 0.3 ml propylene glycol, on the other hand in 4 ml micellar solution. In the same way 1 mg aldrin containing 1 μCi ^{14}C-aldrin was administered in 0.3 ml propylene glycol and in 0.3 ml triolein. Resp. at 1, 3, 6 and 12 hr after administration of the mentioned doses of DDT and aldrin, the rats were anesthetized with ether and samples of perirenal fat and the liver were taken. All samples were stored at - 18 °C and analyzed within 3 days.

6.2.2. Analytical procedures.

Sampling, homogenizing and determination of the amount of radioactivity in perirenal fat and liver samples was performed as mentioned previously (see 2.7.).

6.2.2.1. Determination of DDT and aldrin and their respective metabolites in perirenal fat.

Homogenization and extraction were performed as described in chapter 2 (2.7.1.7. and 2.7.3.). After extraction, the obtained chloroform fraction was purified further with the aid of an aluminium oxide column according to Holden and Marsden (1969). This purification procedure is as follows: The alumina powder used is analytical grade aluminium hydroxyde (Fluka) activated at 800 °C for 4 hr, cooled in a desiccator, partially deactivated by shaking with 5 per cent by weight of distilled water for 30 min and stored in a closed vessel. The column is prepared by placing 2.0 ± 0.1 g of alumina powder in a 45 cm long x 0.6 cm bore chromatographic tube having a glasswool plug at its lower end. For extracts containing up to 100 mg of extracted fat a single alumina column is sufficient for satisfactory clean up. The chloroform extract of the extraction of Bligh and Dyer is evaporated to dryness with N_2 and

the rest taken up in 1.0 ml of hexane. This solution is placed on the alumina surface by means of a pipette. The solution is drained down one side of the glass column and washed into the alumina by refilling the pipette with 1 ml of hexane and draining down the same side of the column. A further 20 ml of hexane is added. After collecting the hexane fractions, the measuring flask is filled with hexane to 25 ml. The column was tested with ^{14}C-DDT and ^{14}C-dieldrin and the activity remaining appeared to be less than 1 per cent. Duplicate 10 ml samples of the hexane solution were brought onto a precoated silica-gel G glass plate as described in 4.2.3. Standard markers of ^{14}C-DDT, ^{14}C-DDD and ^{14}C-DDE were also placed on the plate. Liquid n-heptane according to Kovacs (1963) was used as mobile phase.
The R_f - values for DDT, DDD and DDE were found to be resp. 0.23, 0.11 and 0.37. The R_f - values for aldrin and dieldrin were, respectively, 0.44 and 0.11. The measurement of the radioactivity is described in the general procedures (2.7.4.3.).

6.3. RESULTS

6.3.1. Influence of the dosage form on fat/liver quotients of DDT and aldrin.

As can be seen from fig. 50 the fat/liver quotient for DDT and aldrin increases with time. The absolute concentration of these compounds in the liver was found to be fairly constant. The concentration in perirenal fat increased during 12 hr after administration. It can also be seen in fig. 50A and B, that the fat/liver quotient for DDT and aldrin is influenced by the dosage form employed. As compared with the administration of these compounds in propylene glycol, it appears that a fatty dosage form, probably by favouring the transport via the lymphatics, results in a relatively greater concentration in the fat tissues as compared with the liver (at 12 hr: for DDT and aldrin $P < 0.05$; Student t-test). As mentioned already Norman and DeLuca (1963) found that vitamin D_3, although transported via the lymphatics, accumulates in the liver. This is not the case with DDT, which accumulates to a considerable extent in fat tissue. This might be explained on the basis of our findings in chapter 5, that DDT in a considerable amount is present in the core of the chylomicrons. The core consists of triglyceride which is mainly removed by lipolysis in the fat tissues. So relatively high concentrations of DDT are reached there.

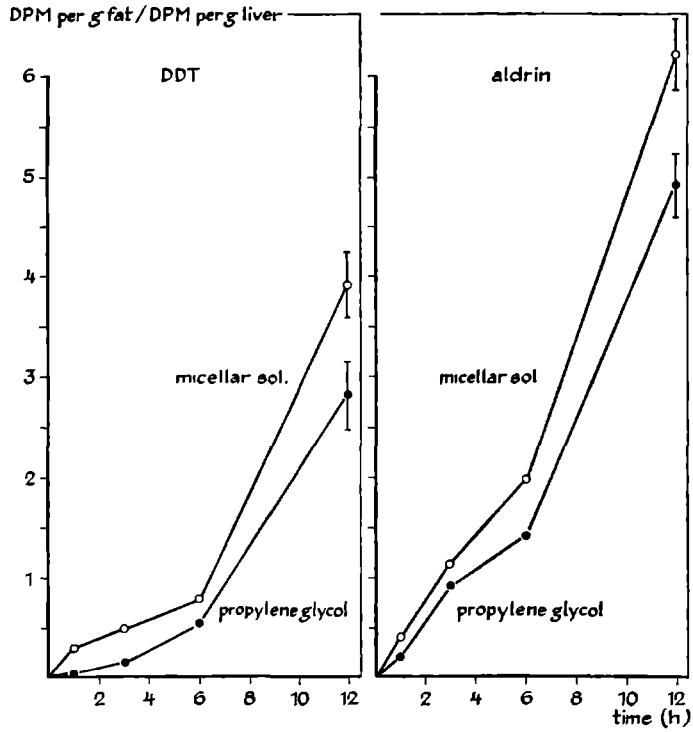


Fig 50 A, B
Fat/liver quotients for ^{14}C-DDT and ^{14}C-aldrin at different times after oral administration in fatty and non-fatty dosage forms The values are based on amounts of total radioactivity It has not been accounted for the presence of metabolites. The vertical bars refer to S E M for 6 animals

6.3.2. Metabolites of DDT and aldrin in perirenal fat.

DDT.

In each experiment 2 samples of perirenal fat were taken at 12 hr after administration. In 1 sample total ^{14}C radioactivity per g was determined. In the other sample, DDT and its metabolites were separated and identified. Two metabolites were found, namely DDD and DDE. The sum of ^{14}C radioactivities of these 3 fractions appeared to be 97 per cent of the radioactivity measured in the former sample. Fig. 51 clearly demonstrates that, depending on a fatty or non-fatty administration form, the ratio between DDT and its metabolites are different. DDT, when administered in propylene glycol, is metabolized to a greater extent than

when it is administered in a micellar solution. This is understandable if we take into account the fact that DDT in propylene glycol is transported to a greater extent via the liver, the main site of metabolism. As mentioned already, the conversion of DDT into DDE by the rat is of minor importance (Peterson and Robison, 1964). In agreement with this the amount of DDE in the renal fat is only 3-4 per cent.

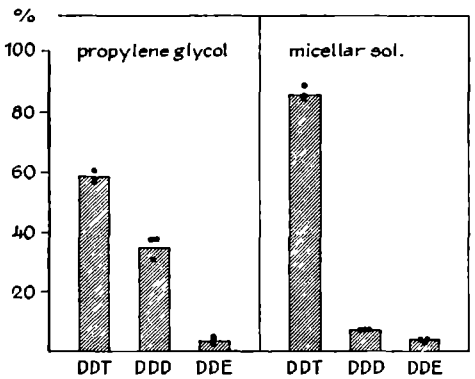


Fig. 51
Amounts of DDT, DDD and DDE in perirenal fat as percentages of total ^{14}C radioactivity, measured at 12 hr after administration of DDT in non-fatty and fatty dosage forms. Means are given for 3 separate experiments.
Note: DDT is metabolized to a greater extent, when administered in propylene glycol. This corresponds with a greater passage through the liver.

Aldrin.
From the literature it is known that aldrin is very rapidly converted to dieldrin in the rat. Therefore, in the case of aldrin administration in the different dosage forms, samples of perirenal fat were taken after 3 hr. Although the lymphatic transport of aldrin, given in triolein, is greater than when administered in propylene glycol, in absolute terms the amount of aldrin which is absorbed via the lymph, is only small compared to DDT. This phenomenon, and in addition the fast metabolism of aldrin, may be the explanation for the smaller difference observed between aldrin and dieldrin in relation to the fatty and non-fatty dosage form (see fig. 52).

6.4. CONCLUSION

The finding that administration of DDT and aldrin in a fatty dosage form results in a relatively greater concentration of these compounds in the fat tissue, may justify the conclusion that the absorption route, which in its turn is dependent upon the dosage form, can influence the distribution pattern of highly lipophilic pharmaca in the organism. In this respect, if transport via the lymph tract occurs, the binding to

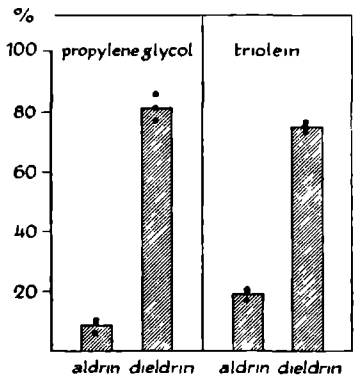


Fig 52
Amounts of aldrin and dieldrin in perirenal fat as percentages of total ^{14}C radioactivity, measured at 3 hr after administration of aldrin in non-fatty and fatty dosage forms Means are given for 3 separate experiments

lipoproteins, particularly the chylomicrons, might also be of importance. On this basis the different distribution pattern of DDT and vitamin D_3 (Norman and DeLuca, 1963) between liver and fat tissue can be explained (see 6 3 1.). Moreover the different routes of absorption dependent on the different dosage forms, has, as a matter of fact, consequences for the biotransformation of lipophilic pharmaca. This is clearly demonstrated in fig. 51, where, if DDT is forced by the non-fatty dosage form to take the absorption route via the liver, then a substantial amount of metabolites is found. However, in the case of transport via the lymphatics as a consequence of the fatty dosage form, practically no metabolites have been observed. It is generally assumed that DDT is metabolically stable. This is probably due to the fact that DDT – since it is in most cases absorbed from the gut in combination with fatty components – does not reach the liver, rather than that this organ is unable to convert it.

CHAPTER 7

SUMMARY

In the present thesis investigations are described which were carried out in order to obtain more information about the role of the lymphatic system in the absorption of pharmaca from the gut The various studies concentrate particularly on the following questions, which are more extensively discussed in **chapter 1:**

- Which drugs are absorbed to a great extent via the lymphatics and what are the properties that lead to such an absorption?
- Is it possible to directly influence the absorption in the direction of the portal system or the lymphatics by means of the vehicle?
- Are there pharmacologic consequences of influencing the route of absorption?

Since the flow of mesenteric lymph is only 1/500 of the flow of the blood in the portal system, it is clear then that absorption of pharmaca via the lymphatics plays only a major role when a concentration of the pharmacon is reached in the lymph which is considerable compared to the blood concentration. A low degree of interchange of the drug between the blood and lymph, may be mentioned as another condition for selective lymphatic transport If a drug is absorbed from the gut via the portal system then it goes directly to the liver, which has a great capacity for metabolizing, and eventually for inactivating, such a compound If, however, a drug is absorbed exclusively via the lymphatic tract of the gut, then this drug will be transported into the blood of the vena subclavia and may reach its site of action without passing through the liver This may of course have pharmacologic consequences

Chapter 2 reports the materials used, the animal experiments and the analytical procedures Special attention was paid to the description of the techniques for lymphatic cannulations and the cannulation of portal vessels The latter procedure was developed in order to obtain samples

of portal blood continuously during several hours whithout anaesthetizing of the rats

In **chapter 3** are described the results which indicate that the dosage form can influence the route of absorption of a drug, either via the portal system or via the lymph tract
The following dosage forms of DDT have been compared in this respect The non-fatty solvent propylene glycol and the fatty solvents triolein and a micellar mixture If DDT was administered in a non-fatty solvent such as propylene glycol, considerably less absorption via the lymphatics took place as compared to DDT administered in the fatty dosage forms However, DDT administered in propylene glycol was to a greater extent absorbed via the portal system So it is clear that DDT, which is highly lipoid soluble, is not only absorbed well from a fatty dosage form but also from a non-fatty solution, be it via an alternative route
From experiments with bile cannulation it might be concluded that, with respect to a selective transport via lymphatics, the micellar solution is preferable to the triolein solution in those cases in which bile flow is disturbed or absent

In **chapter 4** investigations are described concerning the lymphatic transport of 21 compounds including some insecticides, halogenated hydrocarbons, a carcinogen such as methylcholanthrene and other pharmaca On the basis of the results from chapter 3 it could be expected that lymphatic transport would be optimal when these compounds were administered in a fatty dosage form The amount of the various substances (radioactively labelled) were collected in the thoracic duct lymph and measured at different intervals up to 24 hr after oral administration
As to the question posed above regarding which properties of the compounds lead to a considerable lymphatic absorption, it can be said that only those compounds with a high lipophilicity are absorbed selectively via the lymph tract This selective absorption can also be inferred from the great concentration of the compounds in the mesenteric lymph fluid compared with the concentration in the portal blood For the compounds under investigation the correlation between lymphatic transport and lipophilicity was studied As a measure of lipophilicity ΔR_m in respect of butter yellow (4 2 5) was determined with the aid of reversed phase chromatography It appeared that there exists a linear correlation

($r = 0.850$) between the log of the apparent lymphatic absorption and the ΔR_m value of the various compounds.

Chapter 5 reports binding studies with vitamin D_3, DDT, dieldrin, quinestrol and mestranol to lymph components under various conditions. Thoracic duct lymph was collected and fractionated by means of ultracentrifugation into the following sub-fractions: chylomicrons, VLDL (very low density lipoproteins), LDL-HDL (low density lipoproteins and high density lipoproteins) and a fraction containing the other proteins. Three types of binding studies were performed. The pharmacon was administered beforehand to the animal, either per os or intravenously and lymph was sampled and subsequently analyzed or, in the third type of experiments lymph was sampled and thereafter the pharmacon was added in vitro and the lymph was analyzed.
The main result appeared to be that in most cases the investigated pharmaca were present for more than 80 per cent in the chylomicron and other lipoprotein fractions. Binding to other proteins played a minor role. It appeared that the binding per unit mass of the chylomicrons and other lipoproteins was substantially greater when the compounds were administered orally as compared to the binding after intravenous or in vitro application.
In further experiments chylomicrons were subdivided into a nucleus fraction and a shell fraction. The binding of the pharmaca to these fractions has also been studied. It transpired that, particularly after oral administration, the pharmaca enter into the nuclei of the chylomicrons. These results lead to the supposition that there might be a special mechanism operative following oral administration through which these pharmaca are more strongly bound to the chylomicrons and other lipoproteins. It looks as if, following in vitro incubation or intravenous administration, the pharmacon binds to the shell to a larger extent, due to apposition with the outside of the completed chylomicron. However after oral administration the pharmacon may have access to the nucleus to a higher degree.

In **chapter 6** the consequences are described of fatty or non-fatty dosage form for the distribution and metabolism of the insecticides DDT and aldrin. As already mentioned, an initial passage through the liver is avoided when a drug is absorbed selectively via the lymphatics. As has been demonstrated in chapter 3, selective absorption via lymphatics after oral administration of a compound can be obtained

when it is given in a fatty dosage form. On the other hand when a non-fatty dosage form is used the absorption from the gut mainly occurs via the portal system. From the results presented here it appears, that, if DDT is forced (by the non-fatty dosage form) to take the absorption route via the liver, then a considerable amount of metabolite is found. However practically no metabolite was found when transport via the lymphatics occurs. These findings support the idea that differences in the absorption route may have far-reaching consequences for the metabolism and distribution, and in its turn the pharmacologic action, of a compound.

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CURRICULUM VITAE

De auteur werd geboren te Utrecht op 21 augustus 1930. Hij bezocht het St. Franciscuscollege te Rotterdam en behaalde aldaar het diploma H.B.S.-B in 1949. Van 1949 tot 1958 studeerde hij aan de Rijks Universiteit te Utrecht. Gedurende deze periode behaalde hij het candidaatsexamen in de wis- en natuurkunde, onderdeel chemie, in 1953, het doctoraalexamen in de farmacie, met de bijvakken farmacologie en fysiologische chemie in 1957 en de apothekersexamens I en II in het daaropvolgende jaar. Na enige tijd werkzaam te zijn geweest als beherend apotheker in Amsterdam vestigde hij in 1960 een apotheek in Wijchen. In 1969 werden de werkzaamheden voor deze dissertatie aangevangen aan het Farmacologisch Instituut van de Katholieke Universiteit te Nijmegen onder leiding van Prof. Dr. E. J. Ariëns.

STELLINGEN

I

Het lymfatische systeem van de darm speelt een belangrijke rol bij de resorptie van een aantal vetoplosbare geneesmiddelen Dit kan in een aantal gevallen belangrijke farmacologische consequenties hebben

Dit proefschrift

II

De langdurige opslag van DDT in het lichaamsvet van mens en dier is waarschijnlijk voor een belangrijk deel te wijten aan het onder andere met de grote lipofiliteit van dit insecticide samenhangende resorptie- en distributiepatroon en minder aan de inertheid van DDT ten aanzien van het levermetabolisme

Hayes W J, in Drill s Pharmacology in Medecine (DiPalma J R ed) p 994 Mc Graw - Hill Book Cp, New York (1965)
Dit proefschrift

III

De door Levine op grond van experimenten met een drietal oraal toegediende quaternaire ammoniumverbindingen getrokken conclusie, dat "the degree of lipoid solubility may not determine the proportion of absorbed material leaving via the lymph", is alleen geldig voor pharmaca met een relatief lage lipofiliteit

Levine R R, Marco T J de and Dizon C M, 'The lymphatic system its role in the absorption and distribution of drugs , in 4th Intern Congr on Pharmacol, Basel 1969, vol II (Eigenmann, ed), Schwabe and Co, Publishers Basel (1969)
Dit proefschrift

IV

De bevindingen van Giannina et al, dat na orale toediening in sesamolie, al of niet gecombineerd met monooleïde, de ductus thoracicus lymfe van de rat slechts ongeveer 0,4 procent van het toegediende ethinyloestradiol bevat, maar daarentegen tot ongeveer 16 procent van het toegedinede quinestrol, worden verklaard door aan te nemen, dat quinestrol gebonden wordt (chemisch of fysisch) aan vetzuren en/of

monoglycerides. Op grond van de resultaten van dit proefschrift blijkt echter dat de mate van lipofiliteit van quinestrol in eerste instantie bepalend is voor diens opname in chylomicronen en andere lipoproteïnen en vervolgens voor het transport via de lymftractus.

Giannina T., Steinetz B. G. and Meli A., Intern. J. of Fertility **12**, 155-157 (1967).
Dit proefschrift.

V

De isolatie van β - adrenerge receptoren zoals beschreven door Lefkowitz roept twijfels op met betrekking tot de identificatie van de geïsoleerde, catecholamines bindende fractie als β - adrenerge receptoren.

Lefkowitz R. J., Haber E. and O'Hara D. Proc. Nat. Acad. Sci. **69**, 2828-2832 (1972).

VI

De bewering dat bij de secretie van maagzuur onder alle omstandigheden het histamine als intermediair in het geding zou zijn is aan twijfel onderhevig.

Hirschowitz B. I. and Sachs G. Fed. Proc. **27**, 1318-1321 (1969).
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VII

In acht genomen de activiteiten van de apotheker in het kader van de geneesmiddelenvoorziening is opname in de farmaceutische studie van instructies betreffende administratie en economie gewenst.

VIII

De recente gebeurtenissen inzake Vietnam tonen opnieuw aan, dat de strikte onpartijdigheid van een hulpverlenende instantie, zoals het Rode Kruis, wezenlijk is voor een adequate hulpverlening.

IX

Met betrekking tot de risico's voor het milieu en de gevaren verbonden aan het gebruik van DDT dient men voor- en nadelen van het gebruik van deze stof van geval tot geval goed tegen elkaar af te wegen. Het blindelings vervangen ervan door andere stoffen, waarvan een grotere veiligheid niet bewezen is, moet worden afgewezen. Het vervangen ervan door stoffen die als veiliger beschouwd worden op grond van een geringere kennis omtrent hun eigenschappen bergt ernstige gevaren in zich.

Deichmann W. B., Arch. Toxikol. **29**, 1-27 (1972).
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X

Bij het stellen van voorschriften met betrekking tot de geneesmiddelenvoorziening dienen de belangen van de patiënt en niet de historische rechten van apotheker en arts als uitgangspunt te gelden. Bij een dergelijke benadering zal de plaats van de apotheker een duidelijk andere worden dan ze in het verleden was.

XI

Het toenemend gebruik van slaapmiddelen en tranquilizers, goeddeels op doktersvoorschrift, vormt een probleem van tenminste gelijke omvang als het probleem van het zogenoemd druggebruik en verdient derhalve minstens zo veel aandacht.

Nijmegen, 15 maart 1973 H. Kilian